ViroKey® SARS-CoV-2 RT-PCR Test v2.0

Instructions for Use

For use under an Emergency Use Authorization Only

For Prescription Use Only

For In Vitro Diagnostic Use

IVD

Rx Only

Vela Operations Singapore Pte Ltd, #05-07 The Kendall,
50 Science Park Road, Singapore 117406

REF 301068 & 301085

Σ 8x48 tests & 4x96 tests

MAT PS103999F

Consult instructions for use

Version 3.2

Contents

Kit contents

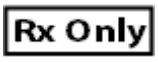
ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

Kit item no.	Item	Cap color	Description	Quantity	Volume / tube
301068	SARS-CoV-2 v2 M1	Green	Mix 1	8	60 µL
	RNA4 M2	Orange	Mix 2	8	700 µL
	RNA4 M3	Pink	Mix 3	8	125 µL
	NC5	Yellow	Negative control (NC)	8	600 µL
	SARS-CoV-2 v2 PC	Blue	Positive control (PC)	8	300 µL
	EC8	Red	Extraction control (EC)	8	600 µL

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

Kit item no.	Item	Cap color	Description	Quantity	Amount
301085	HT SARS-CoV-2 M1	Green	Mix 1	4	115 µL
	HT RNA M2	Yellow	Mix 2	4	1400 µL
	HT RNA M3	Blue	Mix 3	4	112 µL
	HT NC	Natural	Negative control (NC)	4	300 µL
	HT SARS-CoV-2 PC	Blue	Positive control (PC)	4	300 µL
	HT EC	Red	Extraction control (EC)	4	1200 µL

Symbols

	Contains reagents sufficient for <N> tests
	Expiration date
	*In vitro* diagnostic medical devices
	Prescription device
	Catalog number
	Component
	Number
	Content
	Lot number
	Control
	Negative control
	Positive control
	Document / label identification number
	Temperature limitations
	Legal manufacturer
	Refer to instructions for use

Storage

The components of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 should be stored at −20°C ± 5°C and are stable until the expiration date stated on the label. RNA4 M3 / HT RNA M3 is an enzyme, which is in liquid state. Except RNA4 M3 / HT RNA M3, all reagents should be thawed completely before use.

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

Kit item no.	Item	Quantity	Volume / tube	Shipping Condition	Storage Condition
301068	SARS-CoV-2 v2 M1	8	60 µL	Dry ice	-25°C – -15°C
	RNA4 M2	8	700 µL	Dry ice	-25°C – -15°C
	RNA4 M3	8	125 µL	Dry ice	-25°C – -15°C
	NC5	8	600 µL	Dry ice	-25°C – -15°C
	SARS-CoV-2 v2 PC	8	300 µL	Dry ice	-25°C – -15°C
	EC8	8	600 µL	Dry ice	-25°C – -15°C

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

Kit item no.	Item	Quantity	Volume / tube	Shipping Condition	Storage Condition
301085	HT SARS-CoV-2 M1	4	115 µL	Dry ice	-25°C – -15°C
	HT RNA M2	4	1400 µL	Dry ice	-25°C – -15°C
	HT RNA M3	4	115 µL	Dry ice	-25°C – -15°C
	HT NC	4	300 µL	Dry ice	-25°C – -15°C
	HT SARS-CoV-2 PC	4	300 µL	Dry ice	-25°C – -15°C
	HT EC	4	1200 µL	Dry ice	-25°C – -15°C

Intended use

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples collected from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263a, that meet requirements to perform high complexity tests. The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is only for use under the Food and Drug Administration's Emergency Use Authorization.

The following indication is authorized under the FDA's Pooling and Serial Testing [Amendment Letter] for use in laboratories certified under CLIA to perform high complexity tests. The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 test is intended for the qualitative detection of RNA from the SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 3 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, and placed in individual vials containing transport media when tested at least once per week as part of a serial testing program.

This indication is authorized with the testing guidelines within this Instructions For Use. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be reported as presumptive positive or tested individually prior to reporting a result.

Warnings and Precautions

- This test is for use under an Emergency Use Authorization.
- For Prescription Use Only.
- For *in vitro* diagnostic use only (IVD).
- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform high complexity tests.
- Testing of pooled samples is limited to laboratories certified under CLIA, 42 U.S.C. §263a, that meet requirements to perform high complexity tests only.
- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C.§360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The product is to be used by qualified and trained clinical laboratory personnel only.
- Strict compliance with the Instructions for Use is required for optimal PCR results.
- For more information about n=3 pooling strategy for implementation and monitoring see the IFU before proceeding with the PCR workflow.
- Each tube of reagent is designed for 48 reactions.
- Do not use expired kit components. Expiration dates are printed on the box and labels of all components. RNA4 M3 / HT RNA M3 is an enzyme, which is in liquid state. Except RNA4 M3 / HT RNA M3, the rest of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 components should be thawed completely at room temperature (approximately 15°C – 25°C) for up to 30 minutes before use.
- RNA4 M3 / HT RNA M3 should be used directly out of the freezer or kept on ice when performing reagent preparation. Handle carefully to avoid contamination and store the remaining RNA4 M3 / HT RNA M3 immediately after use at ⩽ −20°C for subsequent reactions.
- All reagents, except RNA4 M2 / HT RNA M2 and RNA4 M3 / HT RNA M3, require thorough mixing by quick vortex. Mix RNA4 M2 / HT RNA M2 and RNA4 M3 / HT RNA M3 by gentle inversion. Centrifuge all tubes briefly to collect the contents at the bottom of the tubes. Avoid foaming of the reagents.
- All relevant documents (refer to “Resources” section) should be read thoroughly before performing the assay.
- Mutations that arise within the highly conserved regions of the viral genome covered by the kit’s primers and / or probes may result in failure to detect the presence of the virus.
- May cause allergic skin reactions.

- May be harmful if swallowed.
- Use personal protective equipment as required.
- For additional information, please refer to the Material Safety Data Sheet (MSDS).
- All samples and waste should be considered potentially infectious. Clean and disinfect all work surfaces thoroughly with disinfectants recommended by local authorities.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.
- Clean and decontaminate work area and instruments, including pipettes, with commercially available decontamination products.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipette tips.
- To avoid environmental contamination by amplicons, do not remove the PCR seal after amplification.
- Wash hands thoroughly after handling biological samples and kit reagents.

Safety information

- When working with samples and chemicals, always wear a suitable lab coat, disposable gloves, protective goggles and mask. For more information on the ViroKey® SX Virus Total Nucleic Acid Kit (4x48), ViroKey® HT Virus Total Nucleic Acid Kit (4x96), and the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) and ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96), please refer to the respective material safety data sheets (MSDSs).
- For more safety information on the instruments, please refer to the relevant instrument user manual.
- Discard samples and waste according to local safety regulations.

Quality control

In accordance with Vela Diagnostics' ISO 13485-certified Quality Management System, each lot of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is tested against predetermined specifications to ensure consistent product quality.

Introduction

The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 comprises a ready-to-use system for the detection of SARS-CoV-2 RNA extracted with the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) on the *Sentosa*® SX101 alone, *Sentosa*® SX101 and Thermo Fisher™ KingFisher™ Flex instrument combination (hereinafter known as KingFisher); or SARS-CoV-2 RNA extracted with ViroKey® HT Virus Total Nucleic Acid Kit (4x96) on the Hamilton Microlab® STAR™ instrument. SARS-CoV-2 RNA detection is done via reverse transcription PCR (RT-PCR) on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System or the *Sentosa*® SA201 Real-Time PCR Instrument (hereinafter known as *Sentosa*® SA201, and references to *Sentosa*® SA201 in the user manual will also be applicable to the Applied Biosystems® 7500 Fast Dx Real-Time PCR System for brevity unless otherwise stated).

The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 has specific primers and probes that target the *ORF1a* (FAM reporter dye) and *N* (HEX reporter dye) gene sequences of RNA from SARS-CoV-2 virus for direct detection of the specific amplicons in the same RT-PCR reaction. The genes are detected in the green and orange fluorescence channels respectively, on the *Sentosa*® SA201 Real-Time PCR System.

In addition, the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 contains a third set of primers and probe designed to detect an extraction control (EC) target in the red fluorescence channel. This extraction control is a non-human synthetic DNA fragment added to all samples to control for the nucleic acid extraction steps, and also function as a PCR inhibition control. The EC amplification system does not compromise the detection limit of the analytical SARS-CoV-2 PCR. The test also contains a negative control (NC5 / HT NC) and a positive control (SARS-CoV-2 v2 PC / HT SARS-CoV-2 PC) that allows the user to assess whether the RT-PCR reaction performed correctly. The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 does not cross-react with pathogens that cause similar symptoms, such as influenza.

Principle

The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 uses TaqMan® probe chemistry for real-time RT-PCR detection of viral nucleic acid extracted from respiratory specimens using the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) on an automated workflow using the liquid handler *Sentosa*® SX101 or *Sentosa*® SX101 and Thermo Fisher™ KingFisher™ Flex instrument combination; or the ViroKey® HT Virus Total Nucleic Acid Kit (4x96) on an automated workflow using the liquid handler Hamilton Microlab® STAR™ instrument. The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 contains two primer/probe sets that target the *ORF1a* (FAM reporter dye in the Green fluorescence channel) or *N* (HEX reporter dye in the Orange fluorescence channel) gene sequences of RNA from SARS-CoV-2 virus. The assay also contains primers and a probe to detect an extraction control (EC) sequence, which is a non-human synthetic DNA fragment added to each sample (reporter dye in the Red fluorescence channel).

Nucleic acids extracted from specimens are reverse transcribed into cDNA, and cDNA sequences that are complementary to the oligonucleotide primers are amplified by polymerase chain reaction using the supplied enzyme mixes (tubes RNA4 M2 and RNA4 M3; or tubes HT RNA M2 and HT RNA M3) on the *Sentosa*® SA201 Real-Time PCR system with the *Sentosa*®

SA201 Reporter software. If the target nucleic acids are present and amplified, the probe(s) will anneal to specific complementary sequences located between the corresponding forward and reverse primers during the PCR process. During the extension phase of the PCR, the 5' nuclease activity of DNA polymerase degrades the probe bound to the specific target, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Probes specific to each target generate a fluorescent signal at different wavelengths, enabling the instrument to differentiate between the signals. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the *Sentosa*® SA201 Real-Time PCR system with the *Sentosa*® SA201 Reporter software or the Applied Biosystems 7500 Fast Dx Real-Time PCR System with ABI SDS Software 1.4.1.

Pathogen information

Coronaviruses, which are RNA viruses from the *Coronaviridae* family that are part of the *Coronavirinae* subfamily, cause infectious diseases that mainly infect the respiratory tract, resulting in upper respiratory tract infections (e.g. common cold) in humans[1]. Other symptoms include rhinitis, cough, sore throat, and fever[2].

Previously, six coronaviruses that can infect humans were identified—HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV)[3]. In December 2019, a novel coronavirus was discovered in Wuhan, China. The newly discovered coronavirus is the causative agent of the COVID-19 disease. Human-to-human transmission of the virus via respiratory droplets has been confirmed[4]. In February 2020, the International Committee on Taxonomy of Viruses named the novel coronavirus SARS-CoV-2[5].

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 does not include an Internal Control for RNA extraction/recovery. A known SARS-CoV-2 positive sample should be tested with every batch of patient specimens to monitor the integrity of these process steps.
- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 was validated with nasopharyngeal swabs. Nasal (self-collected under supervision of, or healthcare provider collected), mid-turbinate, and oropharyngeal swab specimens as well as nasopharyngeal wash/aspirate or nasal aspirate specimens, as well as bronchoalveolar lavage samples are also considered acceptable specimen types, but performance has not been established.

- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is validated for use for the following combination of extraction kit, instruments and PCR system:

	Extraction Instrument			Real Time PCR System
Extraction Kit	***Sentosa*® SX101**	**KingFisher**[i]	**Hamilton**[ii]	***Sentosa*® SA201**[iii]
ViroKey® SX VTNA[iv]	✓	✓	✓	✓

- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 has not been evaluated for patients receiving intranasally administered influenza vaccine.
- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 may cross-react with human Adenovirus 71 and *Mycoplasma pneumoniae.*
- Negative results do not preclude SARS-CoV-2 virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported, or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence rates. Positive test results are more likely to represent false positive results during periods of little/no SARS-CoV-2 activity when disease prevalence is low. False negative test results are more likely when prevalence of disease caused by SARS-CoV-2 is high.
- ***N = 3 Pooling:***
  - Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing.
  - Specimens included in pools with a positive or invalid result must be reported as presumptive positive or tested individually prior to reporting a result. Individuals included in a pool that returns a positive or invalid result should be treated as a presumptive positive unless or until they receive a negative result when re-tested individually.
  - However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, they should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result.
  - Specimens with low viral loads may not be detected with pooled testing due to decreased sensitivity or increased interference from pooled testing.

[i] *Sentosa*® SX101 and Thermo Fisher™ KingFisher™ Flex instrument combination
[ii] Hamilton Microlab® STAR™ instrument
[iii] *Sentosa*® SA201 Real-Time PCR system
[iv] ViroKey® SX Virus Total Nucleic Acid Kit (4x48)

- Do not use any reagent past the expiration date, as this may affect performance of the assay.
- Optimum specimen types and timing for peak viral levels during infections caused by a SARS-CoV-2 virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus.
- If SARS-CoV-2 virus mutates in the rRT-PCR target region, the specific novel virus may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was performed.
- The potential for the epidemiology and pathology of disease caused by a specific novel SARS-CoV-2 virus to affect test performance is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 viruses are the causative agent for clinical symptoms.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The performance of this assay has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This assay cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and other authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.
- However, to assist clinical laboratories using the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

  (A) Authorized laboratories[1] using your product will include with test result reports, all

authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

(B) Authorized laboratories using your product will use your product as outlined in the authorized labeling. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

(C) Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

(D) Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

(E) Authorized laboratories using specimen pooling strategies when testing patient specimens with the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 must include with negative test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing."

(F) Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Specimen pooling strategy for implementation and monitoring" recommendations available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

(G) Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Testing Strategies. For the first 12 months from the date of their creation, such records will be made available to FDA within 48 business hours for inspection upon request, and will be made available within a reasonable time after 12 months from the date of their creation.

(H) Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to Vela Diagnostics USA, Inc. through email: support.us@veladx.com or at 877.593.7528 (in the U.S.) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

(I) All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

(J) Vela Diagnostics, authorized distributors, and authorized laboratories using ViroKey® SARS-CoV-2 RT-PCR Test v2.0 will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Controls

A tube of Negative Control (NC), which is nucleic acid-free water, is included in the kit for each run.

A tube of Positive Control (PC), consisting of an IVT-RNA fragment that can be amplified by both sets of primer/probes for *Orf1a* and *N* gene, is included in the kit for each run.

An Extraction Control (EC) is spiked into every test sample in every run and is needed to assess the efficacy of the nucleic acid extraction process, as well as test for the presence of inhibitors and validity of a negative result.

The ViroKey® SARS-CoV-2 RT-PCR test does not include an Internal Control for RNA extraction/recovery. A known SARS-CoV-2 positive sample should be tested with every run of patients' specimens to monitor the integrity of these process steps.

Result interpretation

Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing.

Specimens included in pools with a positive, inconclusive, or invalid result must be tested individually prior to reporting a result.

Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

Negativity / Positivity

The Ct ranges to define negativity / positivity for negative control, positive control and samples for the workflows are listed in the table below. If Ct falls within the range, it is defined as positive (+); if Ct falls out of the range or no Ct is obtained, it is defined as negative (-).

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

Fluorescence channel	Expected Ct values					
	Negative control		Positive control		Samples	
	Not detected	Detected	Not detected	Detected	Not detected	Detected
Green (*ORF1a* gene)	< 10.0, > 38.0 or no Ct	10.0 – 38.0	< 15.0, > 32.0 or no Ct	15.0 – 32.0	< 10.0, > 38.0 or no Ct	10.0 – 38.0

Orange (*N* gene)	< 10.0, > 38.0 or no Ct	10.0 – 38.0	< 15.0, > 32.0 or no Ct	15.0 – 32.0	< 10.0, > 38.0 or no Ct	10.0 – 38.0
Red (Extraction Control, EC)	< 23.0, > 32.0 or no Ct	23.0 – 32.0	< 23.0, > 32.0 or no Ct	23.0 – 32.0	< 20.0, > 38.0 or no Ct	20.0 – 38.0

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

Fluorescence channel	Expected Ct values					
	Negative control		Positive control		Samples	
	Not detected	Detected	Not detected	Detected	Not detected	Detected
Green (*ORF1a* gene)	< 10.0, > 40.0 or no Ct	10.0 – 40.0	< 15.0, > 32.0 or no Ct	15.0 – 32.0	< 10.0, > 40.0 or no Ct	10.0 – 40.0
Orange (*N* gene)	< 10.0, > 40.0 or no Ct	10.0 – 40.0	< 15.0, > 32.0 or no Ct	15.0 – 32.0	< 10.0, > 40.0 or no Ct	10.0 – 40.0
Red (extraction control, EC)	< 23.0, > 32.0 or no Ct	23.0 – 32.0	< 23.0, > 32.0 or no Ct	23.0 – 32.0	< 20.0, > 40.0 or no Ct	20.0 – 40.0

Result interpretation of samples

Please refer to the table below for result analysis. All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

	Green (*ORF1a*)	Orange (*N*)	Red (EC)	Interpretation
Negative control	-	-	+	Run valid (proceed to PC)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	+	+	+/-	
	-	-	-	
Positive control	+	+	+/-	Run valid (proceed to SARS-CoV-2 positive sample control)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	-	-	+/-	
SARS-CoV-2 positive sample control	+	+	+/-	Run valid (proceed to result interpretation of samples)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	-	-	+/-	
Samples	+	+	+/-	SARS-CoV-2 virus detected*
	-	+	+/-	
	+	-	+/-	
	-	-	+	SARS-CoV-2 virus not detected
	-	-	-	Sample invalid. Sample should be retested. If result is still invalid, a new specimen should be obtained.

*For positive samples, the fluorescence channel Cycling Red may be negative due to competition with the target channels.

Run: Whole run on the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode

Test: Test to which the NC / PC belongs

Sample: Single sample in one well of the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode

Result interpretation of n=3 pooling samples

Please refer to the table below for result analysis. All test controls should be examined prior to interpretation of results. If the controls are not valid, the results cannot be interpreted.

	Green (*ORF1a*)	Orange (*N*)	Red (EC)	Interpretation
Negative control	-	-	+	Run valid (proceed to PC)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	+	+	+/-	
	-	-	-	
Positive	+	+	+/-	Run valid (proceed to SARS-CoV-2 positive sample

control				control)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	-	-	+/-	
SARS-CoV-2 positive sample control	+	+	+/-	Run valid (proceed to result interpretation of samples)
	+	-	+/-	Run invalid. Repeat run.
	-	+	+/-	
	-	-	+/-	
Pooled Samples	+	+	+/-	Presumptive Positive[a,b] (Report pooled result to program administrator with notification that subjects in the positive pool should isolate until receiving a negative result when re-tested individually. Individual should not be cohorted with other individuals who have received a positive or presumptive positive result. Individual samples from all subjects in the pool should be re-tested individually.)[c]
	-	+	+/-	
	+	-	+/-	
	-	-	+	SARS-CoV-2 virus not detected[a,d] (Report pooled result to program administrator and appropriate public health authorities.)[c]
	-	-	-	Sample invalid[b]. Sample should be retested. If result is still invalid, a new specimen should be obtained. (If the result is Negative, report pooled result to program administrator and appropriate public health authorities. If the result is Positive or Invalid, report the result as Presumed Positive to program administrator. A new sample from all subjects in the pool should be run and re-tested individually.)[c]

[a]Presumptive Positive" and "Negative" are the specific result interpretations for samples tested as part of the 3-swab pooling procedure.

[b]Individuals included in a pool that returns a positive or invalid result should be treated as a presumptive positive unless or until they receive a negative result when re-tested individually. However, as most individuals in a positive pool will likely receive a negative result when re-tested individually, they should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result. For serial testing programs, additional confirmatory testing for positive results may also be necessary, if there is a low likelihood of COVID-19, such as in individuals without known exposure to COVID-19 or residing in communities with low prevalence of infection.

[c]Results of follow-up testing are reported to the program administrator and appropriate public health authorities.

[d]Specimens with low viral loads may not be detected with pooled testing due to decreased sensitivity or increased interference from pooled testing. For serial testing programs, additional confirmatory testing for negative results may be necessary, if there is a high likelihood of COVID-19, such as an individual with a close contact with COVID-19 or with suspected exposure to COVID-19 or in communities with high prevalence of infection.

Performance characteristics

Analytical sensitivity

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

The analytical limit of detection (LoD) of ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) was assessed with the automated workflow, starting with sample extraction using the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) on the *Sentosa*® SX101, as well as RT-PCR run on the *Sentosa*® SA201. Serial dilutions of the heat-inactivated SARS-CoV-2 in nasopharyngeal matrix were tested to determine the assay LoD. The preliminary LoD was determined by testing three replicates of inactivated virus dilutions between 2.0 x 10^4 and 50 (GE/mL). The LoD was confirmed by testing at least 20 replicates. If the confirmatory study achieved a positivity of 100%, then a lower concentration was tested (with 20 replicates) until less than 100% positivity was obtained. The overall assay LoD was the lowest dilution giving a final sample detection of ≥ 95% for 20 samples for one of the targets (refer to **Tables 1 and 2**). The overall LoD of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 performed on *Sentosa*® SX101 is 200 GE/mL while the overall LoD of the test performed on the *Sentosa*® SX101 and KingFisher combination is 250 GE/mL.

Table 1. Results from confirmatory LoD study for the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) performed on *Sentosa*® SX101.

Target channel	Genomic equivalents/mL	Detection %	Mean Ct ± SD
ORF1a (Green channel)	200	95% (19/20)	32.27 ± 1.66
N gene (Orange channel)	187	95% (19/20)	32.19 ± 1.90

Table 2. Results from confirmatory LoD study for the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) performed on *Sentosa*® SX101 and KingFisher combination.

Target channel	Genomic equivalents/mL	Detection %	Mean Ct ± SD
ORF1a (Green channel)	250	95% (19/20)	31.22 ± 2.41
N (Orange channel)	250	95% (19/20)	33.65 ± 1.81

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

The analytical limit of detection (LoD) of ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) was assessed with the automated Hamilton workflow, starting with sample extraction using the ViroKey® HT Virus Total Nucleic Acid Kit (4x96) on the Hamilton Microlab® STAR™, as well as RT-PCR run on the *Sentosa*® SA201. Serial dilutions of the heat-inactivated SARS-CoV-2 in nasopharyngeal matrix were tested to determine the assay LoD. The preliminary LoD was determined by testing three replicates of inactivated virus dilutions between 1.0 x 10^3 and 125 (GE/mL). The LoD was confirmed by testing at least 20 replicates. If the confirmatory study achieved a positivity of 100%, then a lower concentration was tested (with 20 replicates) until less than 100% positivity was obtained. The overall assay LoD was the lowest dilution giving a final sample detection of ≥ 95% for 20 samples for one of the targets. (**Table 3**). The overall LoD of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) is 200 GE/mL.

Table 3. Results from confirmatory LoD study for the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) performed on Hamilton Microlab® STAR™

Target channel	Genomic equivalents/mL	Detection %	Mean Ct ± SD
ORF1a (Green channel)	187.5	95% (19/20)	34.02 ± 1.13
N (Orange channel)	200	100% (20/20)	33.88 ± 0.57

Analytical reactivity and specificity

The analytical reactivity and specificity of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 are ensured by the selection of primers, probes and stringent reaction conditions.

Analytical reactivity

To evaluate the analytical reactivity (inclusivity) of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 for SARS-CoV-2, *in silico* analysis was performed on all sequences available on the National Center for Biotechnology Information (NCBI) GenBank and Global Initiative on Sharing All Influenza Data (GISAID) databases. 2,636,603 complete sequences (401,846 from NCBI GenBank and 2,234,757 from GISAID — sequences downloaded on 4th August 2021) were aligned against primers and probes of ViroKey® SARS-CoV-2 RT-PCR Test v2.0. The sequences were aligned with MAFFT (https://mafft.cbrc.jp/alignment/server/).

Out of the 401,846 complete SARS-CoV-2 sequences from NCBI GenBank database as of 4th August 2021,

- *ORF1a* primers and probes had 100% match to 396,292 out of 401,846 sequences (98.61%). For the remaining 5554 sequences (1.39%), the primers and probes have at most 1 mismatch with these sequences.
- *N* gene primers and probes had 100% match to 390,973 out of 401,846 sequences

(97.29%). For the remaining 10,873 sequences (2.71%), the primers and probes have at most 1 mismatch with these sequences.

Out of the 2,234,757 complete SARS-CoV-2 sequences from GISAID database as of 4th August 2021,

- *ORF1a* primers and probes had 100% match to 2,202,254 out of 2,234,757 sequences (98.54%). For the remaining 32,503 sequences (1.46%), the primers and probes have at most 1 mismatch with these sequences.
- *N* gene primers and probes had 100% match to 2,163,247, out of 2,234,757 sequences (96.87%). For the remaining 71,510 sequences (3.2%), the primers and probes have at most 1 mismatch with these sequences.

In silico analysis concluded that ViroKey® SARS-CoV-2 RT-PCR Test v2.0 will detect all analysed SARS-CoV-2 sequences in the NCBI GenBank (n = 401,846) and in GISAID (n = 2,234,757) databases. None of the mismatching sequences showed mismatches with the other target, therefore the inclusivity of the assay is not expected to be affected.

Analytical specificity (in silico)

To evaluate the analytical specificity (cross-reactivity) of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0, *in silico* analysis was performed on pathogens listed in **Table 4**. NCBI BLAST tool was used to check for cross-reactivity of the different primers and probes of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 against the non-redundant nucleotide database. BLAST tool search default parameters were used except for the "organism." The search was limited to using the taxonomy ID (taxid/txid) of the respective pathogen. Each primer and probe were compared against all available genome sequences of a certain taxid.

Table 4. *In silico* analysis for ViroKey® SARS-CoV-2 RT-PCR Test v2.0.

		***In silico* analysis for % identity/homology**					
		N			***ORF1a***		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
Coronavirus 229E	NC_002645.1	No alignment was found			No alignment was found		
Coronavirus OC43	NC_006213.1	No alignment was found			No alignment was found		
Coronavirus HKU-1	NC_006577.2	No alignment was found			No alignment was found		
Coronavirus NL63	NC_005831.2	No alignment was found			No alignment was found		
SARS-coronavirus	NC_004718.3	NA	NA	70%	No alignment was found		
MERS-coronavirus	NC_019843.3	No alignment was found			No alignment was found		
Human adenovirus 2	AC_000007.1	No alignment was found			No alignment was found		
Human adenovirus 5	AC_000008.1	No alignment was found			No alignment was found		
Human adenovirus 54	NC_012959.1	65%	NA	NA	No alignment was found		
Human adenovirus A	NC_001460.1	No alignment was found			No alignment was found		
Human adenovirus B1	NC_011203.1	No alignment was found			No alignment was found		
Human adenovirus B2	NC_011202.1	No alignment was found			No alignment was found		

		***In silico* analysis for % identity/homology**					
		N			***ORF1a***		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
Human adenovirus C	NC_001405.1	No alignment was found			No alignment was found		
Human adenovirus D	NC_010956.1	No alignment was found			No alignment was found		
Human adenovirus E	NC_003266.2	No alignment was found			No alignment was found		
Human adenovirus F	NC_001454.1	No alignment was found			No alignment was found		
Human adenovirus type 1	AC_000017.1	No alignment was found			No alignment was found		
Human adenovirus type 35	AC_000019.1	No alignment was found			No alignment was found		
Human adenovirus type 7	AC_000018.1	No alignment was found			No alignment was found		
Human metapneumovirus	NC_039199.1	No alignment was found			No alignment was found		
Human parainfluenza virus 1	NC_003461.1	No alignment was found			No alignment was found		
Human parainfluenza virus 2	NC_003443.1	No alignment was found			No alignment was found		
Human parainfluenza virus 3	NC_001796.2	No alignment was found			No alignment was found		
Human parainfluenza virus 4a	NC_021928.1	No alignment was found			No alignment was found		
Influenza A virus H1N1	GCF_000865725	No alignment was found			No alignment was found		
Influenza A virus H3N2	GCF_000865085	No alignment was found			No alignment was found		
Influenza A virus H5N1	GCF_000864105	No alignment was found			No alignment was found		
Influenza A virus H7N9	GCF_000928555	No alignment was found			No alignment was found		
Influenza B virus	GCF_000820495	No alignment was found			No alignment was found		
Influenza C virus	GCF_000856665.10	No alignment was found			No alignment was found		
Human Parechovirus	NC_001897.1	No alignment was found			No alignment was found		
Enterovirus (e.g. EV68)	NC_038308.1	No alignment was found			No alignment was found		
Human respiratory syncytial virus	NC_001781.1	No alignment was found			No alignment was found		
Human rhinovirus 1	NC_038311.1	No alignment was found			No alignment was found		
Human rhinovirus 3	NC_038312.1	No alignment was found			No alignment was found		
Human rhinovirus 14	NC_001490.1	No alignment was found			No alignment was found		
Human rhinovirus 89	NC_001617.1	No alignment was found			No alignment was found		
Human rhinovirus C	NC_009996.1	No alignment was found			No alignment was found		
Chlamydophila pneumoniae	NC_002180.1	No alignment was found			No alignment was found		
Haemophilus influenzae	NZ_LN831035.1	65%	NA	NA	NA	NA	60%
Legionella pneumophila	NZ_LR134380.1	65%	68%	NA	67%	NA	56%
Mycobacterium tuberculosis	NC_000962.3	No alignment was found			67%	NA	NA
*Streptococcus pneumonia**	NZ_LN831051.1	**80%**	68%	NA	NA	NA	52%
Streptococcus pyogenes	NC_002737.2	No alignment was found			NA	NA	60%
Bordetella pertussis	NC_018518.1	65%	NA	NA	62%	NA	NA
Mycoplasma pneumoniae	NZ_CP010546.1	No alignment was found			NA	NA	52%
Pneumocystis jirovecii (PJP)	GCF_001477535.1	No alignment was found			NA	NA	52%
*Candida albicans**	GCF_000182965.3	65%	74%	67%	62%	NA	60%

		***In silico* analysis for % identity/homology**					
		N			***ORF1a***		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
Pseudomonas aeruginosa	GCF_000006765.1	65%	NA	NA	67%	NA	52%
Staphylococcus epidermis	GCF_000007645.1	NA	68%	NA	NA	71%	NA
Staphylococcus salivarius	CP013216.1	NA	74%	NA	No alignment was found		
Leptospira borgpetersenii	GCF_000013945	65%	NA	NA	NA	NA	56%
Leptospira interrogans	GCF_000092565	65%	79%	NA	No alignment was found		
*Leptospira santarosai**	GCF_000313175	70%	**89%**	NA	NA	62%	NA
Chlamydia psittaci	NC_017287.1	No alignment was found			No alignment was found		
Coxiella burnetii (Q-Fever)	NC_002971.4	NA	68%	NA	62%	NA	60%
Staphylococcus aureus	NC_007795.1	70%	68%	NA	No alignment was found		
*Klebsiella pneumonia**	GCF_000240185.1	**80%**	**84%**	NA	62%	NA	52%
Corynebacterium diphtheriae	NZ_LN831026.1	65%	68%	NA	NA	NA	56%
*Legionella longbeachae**	GCF_000091785.1	65%	68%	NA	**81%**	67%	56%
Bacillus anthracosis (Anthrax)	GCF_000008445.1	NA	68%	NA	NA	NA	56%
Moraxella catarrhalis	NC_014147.1	**85%**	NA	NA	NA	62%	52%
Neisseria elongata	NZ_CP007726.1	70%	68%	NA	62%	NA	NA
Neisseria meningitidis	NZ_LR134525.1	65%	68%	NA	62%	NA	52%
Pooled human nasal wash - to represent diverse microbial flora in the human respiratory tract*	ZLYEM2C (HMP)	**80%**	74%	67%	**86%**	71%	60%
Bat coronavirus HKU4-1	NC_009019.1	No alignment was found			48%	NA	40%
Bat coronavirus HKU5-1	NC_009020.1	No alignment was found			NA	48%	NA
Bat coronavirus HKU9-1	NC_009021.1	No alignment was found			NA	48%	NA
Scotophilus bat coronavirus 512	NC_009657.1	No alignment was found			NA	NA	40%
Bat coronavirus HKU2	NC_009988.1	No alignment was found			48%	48%	48%
Bat coronavirus 1A	NC_010437.1	No alignment was found			48%	48%	NA
Bat coronavirus HKU8	NC_010438.1	No alignment was found			NA	NA	40%
Bat coronavirus BM48-31/BGR/2008	NC_014470.1	No alignment was found			NA	57%	NA
Rousettus bat coronavirus HKU10	NC_018871.1	No alignment was found			48%	NA	40%
Bat coronavirus CDPHE15/USA/2006	NC_022103.1	No alignment was found			NA	48%	40%
BtMr-AlphaCoV/SAX2011	NC_028811.1	No alignment was found			NA	48%	48%

		In silico analysis for % identity/homology					
		N			*ORF1a*		
Microorganism	**Genbank Acc No.**	**Forward Primer**	**Reverse Primer**	**Probe**	**Forward Primer**	**Reverse Primer**	**Probe**
BtRf-AlphaCoV/HuB2013	NC_028814.1	No alignment was found			NA	48%	NA
BtRf-AlphaCoV/YN2012	NC_028824.1	No alignment was found			NA	57%	48%
BtNv-AlphaCoV/SC2013	NC_028833.1	No alignment was found			NA	48%	40%
Rousettus bat coronavirus isolate GCCD.1 356	NC_030886.1	No alignment was found			NA	48%	NA
NL63-related bat coronavirus strain BtKYNL63-9a	NC_032107.1	No alignment was found			NA	NA	40%
Bat coronavirus isolate PREDICT/PDF-2180	NC_034440.1	No alignment was found			NA	NA	40%

Some forward primers, reverse primers, or probes sequences have significant alignments (>80%) with the sequences and these are highlighted in red in table above. Among those are *Streptococcus pneumonia, Leptospira santarosai, Klebsiella pneumonia, Legionella longbeachae and Moraxella catarrhalis*. These pathogens were further analyzed and no potential cross-reactivity is expected based on this *in silico* analysis, as they all do not have nearby or correctly oriented primers or probe with significant alignment (> 80%) to bi-directionally amplify out a PCR product that can be detected on the ViroKey® SARS-CoV-2 RT-PCR Test v2.0. *In silico* analysis of pooled microflora showed potential for cross-reactivity, therefore wet testing was performed (described below).

Analytical specificity (wet testing)

The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 was further evaluated for cross-reactivity with respiratory pathogens commonly present in human respiratory specimens, non-targeted coronaviruses as well as pooled human nasal wash representing the diverse microbial flora in the human respiratory tract. Purified and quantified nucleic acid of the pathogens were added directly into the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 PCR mix. At least three replicates were tested. All controls performed as expected. The results are presented in **Table 5**.

Table 5. Potential cross-reactivity of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0.

Tested pathogens, strain (RNA)		Sample input	*ORF1a*	*N*	EC
Bacteria	*Haemophilus influenzae*	5x 10^6 copies (1x 10^6 copies/ reaction)	0/3	0/3	3/3
	Mycobacterium tuberculosis, H37Ra		0/3	0/3	3/3
	Streptococcus pneumoniae		0/3	0/3	3/3
	Streptococcus pyogenes Rosenbach		0/3	0/3	3/3
	Mycoplasma pneumoniae		0/3	0/3	3/3
	Legionella pneumophila		0/3	0/3	3/3
	Chlamydophila pneumoniae strain CM-1		0/3	0/3	3/3

Tested pathogens, strain (RNA)		Sample input	*ORF1a*	*N*	EC
	Bordetella pertussis		0/3	0/3	3/3
Virus	Human coronavirus 229E		0/3	0/3	3/3
	Human coronavirus OC43		0/3	0/3	3/3
	Human coronavirus HKU1		0/3	0/3	3/3
	Human coronavirus NL63		0/3	0/3	3/3
	Human metapneumovirus (hMPV)		0/3	0/3	3/3
	Human adenovirus 1, Adenoid 71		0/3	0/3	3/3
	Human parainfluenza virus 2, Greer		0/3	0/3	3/3
	Human parainfluenza virus 3, C243		0/3	0/3	3/3
	Human parainfluenza virus 4a		0/3	0/3	3/3
	Human parainfluenza virus 4b, CH 19503		0/3	0/3	3/3
	Influenza A virus (H3N2), A/Aichi/2/68		0/3	0/3	3/3
	Influenza A virus, A/Cali/07/2009 (H1N1 pdm)		0/3	0/3	3/3
	Influenza B virus, B/Lee/40		0/3	0/3	3/3
	Enterovirus, H		0/3	0/3	3/3
	Human Respiratory syncytial virus, 18537		0/3	0/3	3/3
	Rhinovirus 57, Ch47		0/3	0/3	3/3
Natural human flora – pooled human nasal wash		NA	0/3	0/3	3/3

The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 was also evaluated for ability to detect the SARS-CoV-2 variants of concerns at 3x LoD across three replicates The results are presented in **Table 6**.

Table 6. Reactivity of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0.

SARS-CoV-2 Variants tested	Sample input	*ORF1a*	*N*
Alpha B.1.1.7 variant (Twist Bioscience RNA Control 15)	3x LoD	32.75 ± 1.10	32.46 ± 0.31
Beta B.1.351 variant (Twist Bioscience RNA Control 16)		32.38 ± 0.26	31.92 ± 0.25
Gamma P.1 variant (Twist Bioscience RNA Control 17)		32.29 ± 0.06	32.83 ± 0.28
Delta B.1.617.2 variant (Twist Bioscience RNA Control 23)		31.12 ± 0.05	31.50 ± 0.56

Interfering substances

The objective of the study was to verify the effect of potentially interfering substances on the performance of ViroKey® SARS-CoV-2 RT-PCR Test v2.0. Base pool of SARS-CoV-2 positive samples were prepared with heat-inactivated SARS-CoV-2 virus (from American Type Culture Collection (ATCC® part number VR-1986HK) into negative nasopharyngeal specimens. For each of the interference substances, 10x stock concentrations were added to the SARS-CoV-2 positive samples. The test pool contained the specified test concentrations noted in **Table 7** for each interfering substance. This study demonstrates that the potential interfering substances tested at specific concentrations as stated in **Table 7** have no impact on the performance of ViroKey® SARS-CoV-2 RT-PCR Test v2.0.

Table 7. List of potential interfering substances tested with ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) on *Sentosa*® SX101, *Sentosa*® SX101 and KingFisher Combination, and ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) on Hamilton Microlab® STAR™.

Substance	Active Ingredient(s)	Conc.	Detection % (*ORF1a*)	Detection % (*N*)
Nasal Wash (Flo®)	Sodium chloride, potassium chloride, calcium lactate pentahydrate	15% (v/v)	100% (3/3)	100% (3/3)
Nasal Spray/drops (Nazolin®)	Oxymetazoline HCL	15% (v/v)	100% (3/3)	100% (3/3)
Nasal corticosteroids	Fluticasone	5% (v/v)	100% (3/3)	100% (3/3)
Systemic antibacterial	Tobramycin	4 µg/mL	100% (3/3)	100% (3/3)
Antiviral drugs	Oseltamivir	3.3 mg/mL	100% (3/3)	100% (3/3)
Homeopathic relief (Prospan®)	Extract from ivy leaf (Hedera helix L. leaf), Potassium sorbate, anhydrous citric acid, xanthan gum, cherry flavour, crystallizing sorbitol syrup	5% (v/v)	100% (3/3)	100% (3/3)
Antimicrobial/antiviral/ anesthetic lozenges (Dorithricin®)	Benzalkonium, Benzocaine, Tyrothricin	15% (w/v)	100% (3/3)	100% (3/3)
Whole blood	NA	2% (v/v)	100% (3/3)	100% (3/3)
Mucin		60 µg/mL	100% (3/3)	100% (3/3)
Pooled human nasal wash		NA	100% (3/3)	100% (3/3)

FLUMIST nasal spray flu vaccine was not tested for its potential interference with ViroKey® SARS-CoV-2 RT-PCR Test v2.0.

FDA SARS-CoV-2 reference panel testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method and instrument used were ViroKey® SX Virus Total Nucleic Acid Kit (4x48) and *Sentosa*® SA201 Real-Time PCR Instrument. The results are summarized in **Table 8**.

Table 8. Summary of LoD confirmation result using the FDA SARS-CoV-2 reference panel.

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

Clinical performance

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

The clinical validation study of ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) was conducted on nasopharyngeal swabs from unaltered patient samples initially tested with an FDA authorized molecular assay as a comparator method. All samples were extracted with ViroKey® SX Virus Total Nucleic Acid Kit (4x48) on the *Sentosa*® SX101 instrument or in conjunction with the Thermo Fisher™ KingFisher™ Flex instrument and detected with ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) on the *Sentosa*® SA201.

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) performed on Sentosa® SX101

A total of 68 nasopharyngeal samples were tested and the results are summarized in **Table 9**. The performance of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) on *Sentosa*® SX101 was calculated based on the result interpretation table on page 12, where a sample was considered positive if signals are detected by *ORF1a* and/or *N* target(s), while a sample was considered negative if no signals are detected by both *ORF1a* and *N* targets.

Table 9. Summary of clinical performance results (for *Sentosa*® SX101)

		Comparator Results	
		Positive	**Negative**
ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)	**Positive**	33	0
	Negative	1	34

Positive Agreement: 97.1% (33/34) **95% CI:** 85.1 - 99.5%

Negative Agreement: 100% (34/34) **95% CI:** 89.8 - 100%

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) performed on Sentosa® SX101 and KingFisher combination

A total of 60 nasopharyngeal samples were tested and the results are summarized in **Table 10**. The performance of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) using *Sentosa®* SX101 and KingFisher combination was calculated based on the result interpretation table on page 12, where a sample was considered positive if signals are detected by *ORF1a* and/or *N* target(s), while a sample was considered negative if no signals are detected by both *ORF1a* and *N* targets.

Table 10. Summary of clinical performance results (for *Sentosa®* SX101 and KingFisher combination)

		Comparator Results	
		Positive	**Negative**
ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)	**Positive**	30	0
	Negative	0	30

Positive Agreement: 100% (30/30) **95% CI:** 88.6 - 100%
Negative Agreement: 100% (30/30) **95% CI:** 88.6 - 100%

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

The clinical validation study of ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) was conducted on nasopharyngeal swabs from unaltered patient samples initially tested with an FDA authorized molecular assay as a comparator method. All samples were extracted with ViroKey® HT Virus Total Nucleic Acid Kit (4x96) on the Hamilton Microlab® STAR™ instrument and detected with ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) on the *Sentosa®* SA201.

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) performed on Hamilton Microlab® STAR™

A total of 68 nasopharyngeal samples were tested and the results are summarized in **Table 11**. The performance of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) was calculated based on the result interpretation table on page 12, where a sample was considered positive if signals are detected by *ORF1a* and/or *N* target(s), while a sample was considered negative if no signals are detected by both *ORF1a* and *N* targets.

Table 11. Summary of clinical performance results (for Hamilton Microlab® STAR™ instrument).

		Comparator Results	
		Positive	**Negative**
ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)	**Positive**	30	0
	Negative	0	30

Positive Agreement: 100% (30/30) **95% CI:** 88.6 - 100%
Negative Agreement: 100% (30/30) **95% CI:** 88.6 - 100%

Sample preparation (for specimen pooling)

NOTE: Refer to the *"Sample preparation"* steps for the respective Workflows SX101, KingFisher and Hamilton.

The following upper respiratory tract specimens, authorized under the Emergency Use Authorization of the ViroKey® SARS-CoV-2 RT-PCR Test Kit v2.0, may be tested with sample pooling:

- nasopharyngeal,
- oropharyngeal,
- mid-turbinate and
- nasal swab specimens collected into VTM / UTM.

NOTE: Only specimens collected into VTM / UTM may be combined for each sample pool.

Up to 3 patient samples can be combined into a single pool before RNA extraction. Each RT-PCR reaction plate can contain pools of different numbers of samples (2 – 3 samples) as well as individual samples. The volume of each individual patient sample or sample pool used in RNA extraction is 230 µL.

NOTE: Laboratories are to use their protocols and systems to name and track the samples in pools throughout the workflow.

IMPORTANT: Pools with test results that are positive, invalid or inconclusive require extraction and testing of the individual patient samples.

Combine equal volumes (of up to 3 patient samples) into a single tube to make up a total volume of ≥ 1,200 µL. Example volumes are shown in the following table.

Sample	Volume	
	N* = 3	N* = 2
1	400 µL	600 µL
2	400 µL	600 µL
3	400 µL	–
Total volume	1,200 µL	1,200 µL

* N represents the number of samples used

Store the remaining un-pooled volumes of patient samples according to CDC guidelines.

Specimen pooling strategy for implementation and monitoring

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the:

- positivity rate in the testing population and
- efficiency of the pooling workflow.

NOTE:

- Individual specimens with low viral loads may **not** be detected due to decreased sensitivity or increased interference when tested with pooled testing.
- The ViroKey® SARS-CoV-2 RT-PCR test v2.0 have been validated for n-sample pool sizes up to three samples per pool.

Before implementation of pooling

Determination of appropriate pool size

Before a pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate and desired testing efficiency.

a) If historical laboratory data for individual specimens are available

- If historical data for individual specimens from the previous 7 – 10 days are available, estimate the $P_{individual}$ (percent positivity rate) based on individual results.

  $P_{individual}$ = (Number of positive specimens over chosen date range ÷ Total number of specimens tested over chosen date range) × 100%

- Using the calculated $\mathbf{P_{individual}}$ and **Table 1**, identify the appropriate $\mathbf{n_{maxefficiency}}$ (number of samples to pool for maximum efficiency). If $P_{individual}$ is greater than 25%, pooling of patient specimens will **not** be efficient. Thus, pooling should not be implemented.
- If $P_{individual}$ < 13%, pooling of 3 patient specimens can be tested but pooling efficiency will **not** be maximized.

  NOTE: Pooling with greater than 3 patient specimens has **not** been validated and should **not** be performed.

b) If historical laboratory data for individual specimens are unavailable

- If historical data from the previous 7 – 10 days are **unavailable**, 3-specimen pooling may still be implemented.

  NOTE: Without calculating $P_{individual}$, the pooling size implemented may **not** maximize pooling efficiency.

Table 12. Number of samples to pool based on the percent of positive individuals in the population in order to achieve the maximum efficiency.

P (Percentage of positive individuals in the tested population)	$n_{maxefficiency}$ (Number of samples to pool for maximum efficiency)	F (Efficiency of pooling n samples)
13% – 25%	3	1.48 – 1.10

After Implementation of Pooling

Ongoing Monitoring of Pooling Strategy

a) If historical laboratory data for individual specimens is available

- After implementation of a pooling strategy, evaluate the performance of pooled testing by comparing the percent positivity rate of pooled testing to that of individual testing.
- Calculate the P_{pools} (percent positivity rate among patient specimens during specimen pooling) on a daily basis using a moving average of the data from the previous 7 – 10 days of testing.

  P_{pools} = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools over chosen date range ÷ Total number of patient specimens tested in pools over chosen date range) × 100%

- To ensure the pooling efficiency, it is recommended that P_{pools} be reassessed periodically. P_{pools} should be < 25% while sample pooling is implemented by the laboratory. Otherwise, pooling of patient specimens should be discontinued.

b) If historical laboratory data for individual specimens are unavailable

- After initiating a pooling strategy, evaluate the performance of pooled testing by calculating the $P_{pools\text{-}initial}$ (initial percent positivity rate for pooled specimens).
- $P_{pools\text{-}initial}$ is the percent positivity rate for pooled specimens for the first 7 – 10 days of pooled testing.
- Calculate the $P_{pools\text{-}initial}$ (initial percent positivity rate for individual specimens from pool testing) from the first 7 – 10 days of testing.

  $P_{pools\text{-}initial}$ = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools in first 7 – 10 days ÷ Total number of patient specimens tested in pools in the first 7 – 10 days) × 100%

- If $\mathbf{P_{pools\text{-}initial} > 25\%}$, pooling of patient specimens is **not** efficient and should be discontinued until the percent positivity rate decreases.
- If $\mathbf{P_{pools\text{-}initial} \leq 25\%}$, pooling of patient specimens can be continued.

- Continue to monitor pooling strategy by calculating the $P_{pools\text{-}x}$ (percent positivity rate among patient specimens during specimen pooling) for subsequent periods of 7 – 10 days. $P_{pools\text{-}x}$ should be updated daily using a moving average.
- To ensure the pooling efficiency, it is recommended that $P_{pools\text{-}x}$ be reassessed periodically and should be < 25% while sample pooling is implemented by the laboratory. Otherwise, pooling of patient specimens should be discontinued.

  IMPORTANT:
  - Authorized laboratories must keep records of their sample specimen pooling test result data, daily testing totals including number of pooled test results, number of individuals tested and daily running average of percent positive results, as part of monitoring the pooling strategy.
  - For the first 12 months from the date of their creation, such records must be made available to FDA upon request within 48 business hours for inspection. After 12 months from the date of their creation, upon FDA request, such records must be made available for inspection within a reasonable time.

  NOTE: When resources availability is sufficient to meet testing demand, the FDA recommends that laboratories consider whether the risks of reduced test sensitivity with pooling outweigh the benefits of resource conservation.

Result interpretation and test reports (for pooled specimens)

- The result interpretation for pooled specimen results is the same as individual test results (refer to **Result interpretation** on page 12).
- Authorized laboratories testing pooled specimens with ViroKey® SARS-CoV-2 RT-PCR Test v2.0 must include with test result reports for specific individuals whose specimen(s) were the subject of pooling, a notice that their test result is “presumed positive” unless or until they are re-tested individually if the pool in which they were included returns a positive or invalid result.
- The test reports must include instructions to collect a new specimen to be tested individually and must indicate that such individuals should isolate until receiving a negative result when re-tested individually and should not be cohorted with other individuals who have received a positive or presumptive positive result.

Workflow using ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

Sentosa® SX101 workflow

The workflow starts with sample off-board lysis, followed by the extraction of nucleic acids and RT-PCR set up with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate using the *Sentosa*® SX101.

The ViroKey® SX Virus Total Nucleic Acid Kit (4x48) is used for nucleic acid extraction.

After sample lysis and RT-PCR set-up, the MicroAmp® Fast Optical 96-Well Reaction is sealed and then transferred to the *Sentosa*® SA201 or Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument for PCR amplification followed by data analysis.

The *Sentosa*® SA201 is a rebranded version of the Applied Biosystems® 7500 Fast Dx Real-Time PCR System, thus the workflow is interchangeable between the two instruments. For brevity, only the *Sentosa*® SA201 workflow will be mentioned in these instructions for use.

An overview of the workflow is provided (see ***Sentosa*® SX101 Flowchart**).

Sentosa® SX101 Flowchart: Automated workflow overview

Sample pre-treatment and lysis using ViroKey® SX Virus Total Nucleic Acid Kit (4x48)

Load consumables, reagents, controls and pre-treated samples on the *Sentosa*® SX101

-
-
-
-

Define the extraction and RT-PCR set-up run using the user interface

Start the run

Transfer the 96-Well Reaction Plate into the *Sentosa*® SA201

Define the RT-PCR run using the user interface

Start the run

After the run is completed, analyze the results using the *Sentosa*® SA201 Reporter software (or Fast Dx)

Maintenance

KingFisher workflow

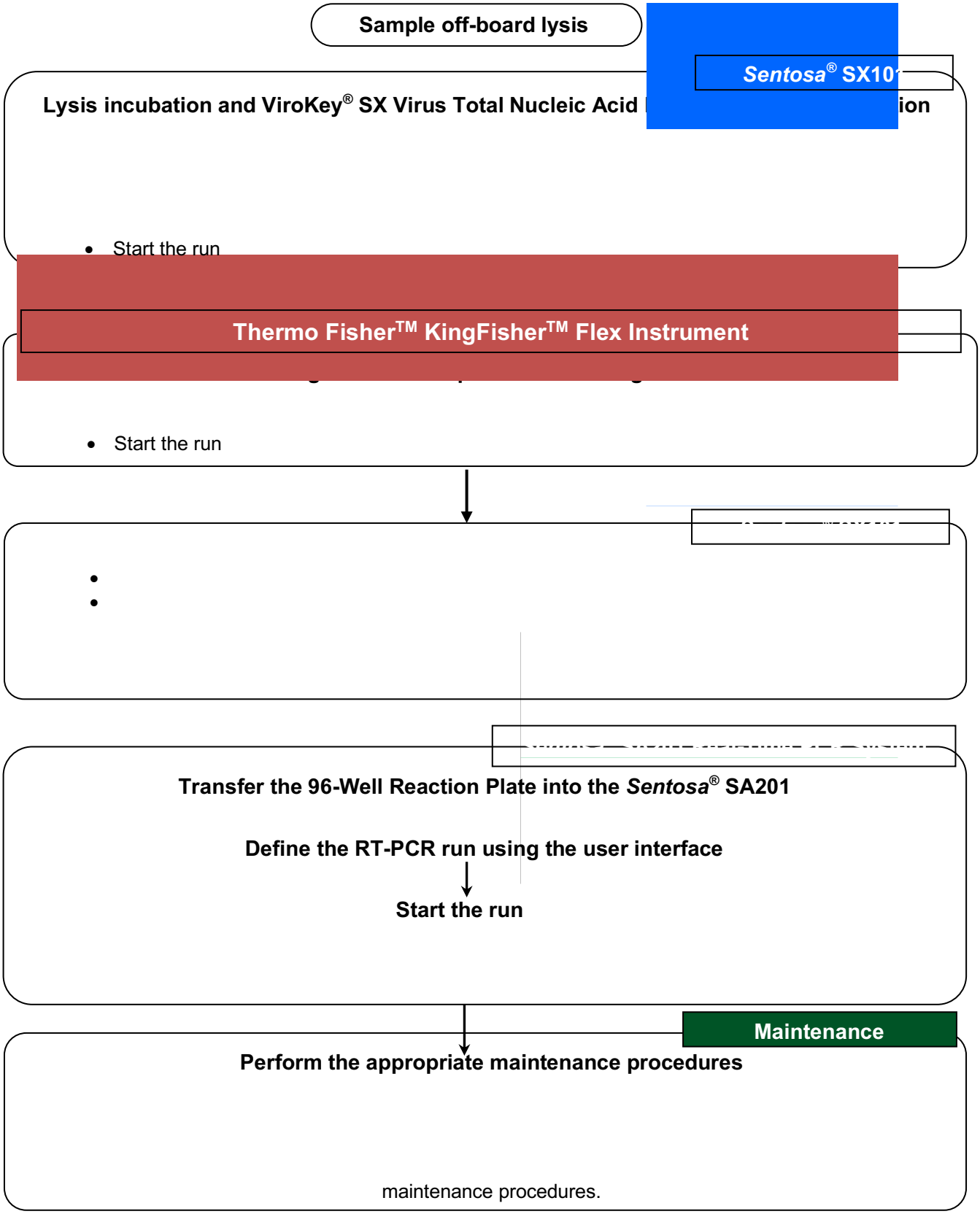
The workflow starts with sample off-board lysis, followed by lysis incubation and ViroKey® SX Virus Total Nucleic Acid Kit (4x48) buffer preparation on the *Sentosa*® SX101, nucleic acid extraction on the Thermo Fisher™ KingFisher™ Flex instrument, and finally RT-PCR set up with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate using the *Sentosa*® SX101.

The ViroKey® SX Virus Total Nucleic Acid Kit (4x48) is used for nucleic acid extraction.

After sample lysis and RT-PCR set-up, the MicroAmp® Fast Optical 96-Well Reaction and then transferred to the *Sentosa*® SA201 PCR amplification respectively. This is followed by data analysis using *Sentosa*® SA201 Reporter or SA Reporter.

An overview of the workflow is provided (see **KingFisher Flowchart**).

KingFisher Flowchart: Automated workflow overview


Sample off-board lysis
Sentosa® SX10
Lysis incubation and ViroKey® SX Virus Total Nucleic Acid
ion
Start the run
Thermo Fisher™ KingFisher™ Flex Instrument
Start the run
Transfer the 96-Well Reaction Plate into the Sentosa® SA201
Define the RT-PCR run using the user interface
Start the run
Maintenance
Perform the appropriate maintenance procedures
maintenance procedures.

Items to be supplied by user

Table 13. List of items to be supplied by user

Equipment / software	Description / use	Vela item no.
Pipettes (adjustable)[i]	For pipetting buffers, reagents and / or samples	N/A
Vortex mixer	To mix reagents	N/A
Bench top centrifuge[i]	To spin down reagents and remove any bubbles	N/A
Thermomixer[i]	To heat and mix samples	N/A
MPS 1000 Mini Plate Spinner, 120V or Eppendorf Centrifuge 5430 / 5430R with Rotor FA-45-24-11	For PCR plate centrifugation	N/A
Sentosa® SX101 instrument[i]	Automated sample processing system	400089
Thermo Fisher™ KingFisher™ Flex instrument with 96 Deep-well Head[i]	Automated sample processing system	400271
Sentosa® SA201 Real-Time PCR Instrument[i]	Real-time and end-point thermal cycling using PCR, detection and analysis	400125
Sentosa® SA201 Series Software	To operate *Sentosa*® SA201 Real-time PCR Instrument and to perform PCR data analysis	460012
Sentosa® SA201 Reporter[i]	To automate data analysis and result interpretation from *Sentosa*® SA201	480142
OR		
ABI 7500 Fast Dx Real-Time PCR instrument	Real-time and end-point thermal cycler for PCR, detection and analysis	N/A
ABI 7500 Fast Dx SDS Software	To operate ABI 7500 Fast Dx Real-Time PCR instrument and to perform manual PCR data analysis	N/A
Accessories / consumables / reagents	**Description / use**	**Vela item no.**
ViroKey® SX Virus Total Nucleic Acid Kit (4x48)[ii]	4x48 tests	300678
Sentosa® SX Magnetic Separator	1 piece	400024
Sentosa® SX Non-Partition 50 µL Filter Tips (960)	10 boxes of 96 tips. Alternatively, tips compatible with Eppendorf TM50/TS50 dispensing tool may be used.	400224

[i] Ensure that the instruments have been checked and calibrated according to the manufacturer's recommendations.
[ii] For nasopharyngeal swabs, use ViroKey® SX Virus Total Nucleic Acid Kit (4x48) for virus total nucleic acid extraction.

Accessories / consumables / reagents	Description / use	Vela item no.
Sentosa® SX Non-Partition 1000 µL Filter Tips (960)	10 boxes of 96 tips. Alternatively, tips compatible with Eppendorf TM1000/TS10000 dispensing tool may be used.	400223
Sentosa® SX 100 mL Reservoir (50)	Set of 50 reservoirs	400027
Sentosa® SX 30 mL Reservoir (50)	Set of 50 reservoirs	400028
Sentosa® SX Deepwell Plate 96/2000 µL (20)	Set of 20 plates	400068
Sentosa® SX Biohazard Bag (100)	100 pieces	400033
KingFisher™ 96-Deepwell Plates (6)	6 plates	400294
KingFisher™ 96-Tip Comb (1)	1 tip comb	400295
Sterile pipette tips with filters	For pipetting buffers, reagents and / or samples	N/A
Safe-Lock Tubes (1000)	For sample extraction. Alternatively, Sarstedt 1.5 mL screw cap tubes (or tubes of equivalent dimensions) may be used.	400031
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL[iii]	N/A	N/A
MicroAmp® Optical Adhesive Film[iv]	N/A	N/A
MicroAmp™ Adhesive Film Applicator[v]	For applying the MicroAmp® Optical Adhesive Film to seal the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode	N/A
Swabs	For collecting nasopharyngeal swab samples	N/A
Absolute ethanol	For adding to Buffer D3 in ViroKey® SX Virus Total Nucleic Acid Kit (4x48)	N/A

[iii] MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL from Applied Biosystems® (Cat. No. 4346906) **MUST** be used. Ensure that the correct plates are used.
[iv] MicroAmp® Optical Adhesive Film from Applied Biosystems® (Cat. No. 4311971) **MUST** be used. Use only unexpired films.
[v] MicroAmp™ Adhesive Film Applicator from Thermo Fisher Scientific (Cat. No. 4333183) is recommended.

Workflow using ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

Hamilton workflow

The workflow starts with sample plate preparation followed by the lysis and extraction of nucleic acids, and RT-PCR set up with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate using the Hamilton Microlab® STAR™.

For nasopharyngeal swabs, use the ViroKey® HT Virus Total Nucleic Acid Kit (4x96) for nucleic acid extraction.

After sample lysis and RT-PCR set-up, the MicroAmp® Fast Optical 96-Well Reaction is sealed, and then transferred to the *Sentosa*® SA201 for PCR amplification respectively. This is followed by data analysis using *Sentosa*® SA201 Reporter or SA Reporter.

An overview of the workflow is provided (see **Hamilton Flowchart**).

Hamilton Flowchart: Automated workflow overvie

amilton Microlab® STAR™

- Start the run.

Viral RNA Extraction

-
-
-
- Start the run.

HT PCR Setup

- Prepare the PCR MM using M1, M2 and M3 from ViroKey® SARS-CoV-2 RT-PCR

Define the RT-PCR run using the user interface

Start the run

Maintenance

Items to be supplied by user

Table 14. List of items to be supplied by user.

Equipment / software	Description / use	Vela item no.
Pipettes (adjustable)[i]	For pipetting buffers, reagents and / or samples	N/A
Vortex mixer	To mix reagents	N/A
Bench top centrifuge[i]	To spin down reagents and remove any bubbles	N/A
Thermomixer[i]	To heat and mix samples	N/A
Hamilton Microlab® STAR™	Automated sample processing system	400316
Sentosa® SA201 Real-Time PCR Instrument[i]	Real-time and end-point thermal cycling using PCR, detection and analysis	400125
Sentosa® SA201 Reporter[i]	To automate data analysis and result interpretation from *Sentosa*® SA201	480142
Accessories / consumables / reagents	**Description / use**	**Vela item no.**
ViroKey® HT Virus Total Nucleic Acid Kit (4x96)	4x96 tests	300678
HT 300 mL Reservoir (40)	To contain the reagents	400298
HT 8-Strip Tubes, Clear, 0.2 mL (125)	8-strip tubes (1 PCR Strip)	400299
HT Reagent Tub with Lid, 60 mL (28)	60 mL Trough	400307
HT U Deepwell Plate, Barcoded, 2.2 mL (24)	Deepwell plate	400308
HT Conductive 1 mL Filter Tips (3840)	For pipetting buffers, reagents and / or samples	400309
HT Conductive 300 µL Filter Tips (5760)	For pipetting buffers, reagents and / or samples	400310
HT Conductive 50 µL Filter Tips (5760)	For pipetting buffers, reagents and / or samples	400311
Sterile pipette tips with filters	For pipetting buffers, reagents and / or samples	N/A
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL[ii]	N/A	N/A
MicroAmp® Optical Adhesive Film[iii]	N/A	N/A
MicroAmp® Adhesive Film Applicator[v]	For applying the MicroAmp® Optical Adhesive Film to seal the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode	N/A
Regular flocked swabs (nasopharyngeal)[vi]	For collecting nasopharyngeal swab samples	N/A
Absolute ethanol	For adding to Buffer D3 in ViroKey® HT Virus Total Nucleic Acid Kit (4x96)	N/A

[i] Ensure that the instruments have been checked and calibrated according to the manufacturer's recommendations.
[ii] MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL from Applied Biosystems® (Cat. No. 4346906) **MUST** be used. Ensure that the correct plates are used.
[iii] MicroAmp® Optical Adhesive Film from Applied Biosystems® (Cat. No. 4311971) **MUST** be used. Use only unexpired films.
[v] MicroAmp™ Adhesive Film Applicator from Thermo Fisher Scientific (Cat. No. 4333183) is recommended.
[vi] BD™ Regular flocked swab, sterile single wrapped (Cat. No. 220250) or BD™ Flexible minitip flocked swab, sterile single wrapped (Cat. No. 220252) in BD™ Universal viral transport, 3 mL vial (Cat. No. 220220) OR 16 X 100 mm Screw Cap Tube containing 3 mL of UTM Transport and Preservation Medium, 1 Nasopharyngeal Flocked Swab (Cat. No. 305C).

Important notes

General precautions

- Use sterile pipette tips with filters.
- During manual steps, ensure that the tubes are closed when possible to avoid contamination.
- **Do not mix components from kits with different lot numbers.**
- Proceed continuously from one part of the workflow to the next.

Specimen collection, handling and storage

- Specimen collection:
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
  - Follow specimen collection device manufacturer instructions for proper collection methods.
- Transporting specimens:
  - Specimens must be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8°C and ship overnight on ice pack. If a specimen is frozen at ≤-70°C, ship overnight on dry ice.
- Storing specimens:
  - After collection and during transport, the specimen should be stored at 2 – 8°C and all laboratory testing must occur within 72 hours of collection. Refrigerated specimens received outside of this 72-hour window should be rejected.
  - If a delay in shipping is expected, store specimens at ≤ -70°C.
  - Specimens received frozen should be stored at ≤ -70°C until processing.
  - Store any residual specimens at ≤ -70°C.

  NOTE: Inadequate specimen collection and / or inappropriate specimen processing, storage and transport may yield false negative results.

Storage of purified nucleic acid

- Purified nucleic acids should be stored at ≤ -70°C.

Protocol: Automated nucleic acid isolation and detection on the *Sentosa*® SA201 / Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

The ViroKey® SX Virus Total Nucleic Acid Kit (4x48) is intended for virus total nucleic acid extraction from respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) and bronchoalveolar lavage samples for use with the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48).

Important points before starting

- User must be familiar with operating the *Sentosa*® SX101 instrument and the *Sentosa*® SA201 / Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument. Please refer to the respective user manuals supplied with the instruments for operating instructions.
- Before beginning the procedure, read the “Important notes” section, page 41.
- Ensure that all reagents of the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) are not precipitated before use. If precipitates are observed, dissolve by incubating in a water bath (≤ 37°C).
- Each tube of reagent in the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) is designed for 48 reactions.

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

The ViroKey® HT Virus Total Nucleic Acid Kit (4x96) is intended for virus total nucleic acid extraction from respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens and nasopharyngeal wash/aspirate or nasal aspirate specimens) and bronchoalveolar lavage samples for use with the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96).

Important points before starting

- User must be familiar with operating the Hamilton Microlab® STAR™ instrument and the *Sentosa*® SA201. Please refer to the respective user manuals supplied with the instruments for operating instructions.
- Before beginning the procedure, read the “Important notes” section, page 41.
- Ensure that all reagents of the ViroKey® HT Virus Total Nucleic Acid Kit are not precipitated before use. If precipitates are observed, dissolve by incubating in a water bath (≤ 37°C).
- Each tube of reagent in the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) is designed for 96 reactions.

ViroKey® SX Virus Total Nucleic Acid Kit (4x48) / ViroKey® HT Virus Total Nucleic Acid Kit (4x96)

- The Mag tubes containing magnetic beads require thorough vortexing for 5 minutes before the start of the workflow to ensure proper re-suspension.
- Prior to use, cRNA (lyophilized carrier RNA) must be reconstituted and aliquoted. Refer to detailed procedure for more information.
- Mix the buffers in the bottles by gentle swirling, ensuring no foam or bubbles are present.

- RNA4 M3 / HT RNA M3 is an enzyme, which is in liquid state. Except RNA4 M3 / HT RNA M3, the rest of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 components should be thawed at room temperature (approximately 15°C – 25°C).

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

- For software, use current version or higher

Sentosa® SX101	Release 3.0 (Version 41.0.1.5)
Sentosa® SA201 Series Software	Version 1.0.1 (for use with *Sentosa*® SA201)
Sentosa® SA201 Reporter	Version 1.5 (for use with *Sentosa*® SA201)
ABI SDS Software	Version 1.4.1 (for manual data analysis with ABI 7500 Fast Dx)

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

- For software, use current version or higher

Hamilton Run Control	Release 4.5.0.5217 (for use with Hamilton Microlab® STAR™)
Sentosa® SA201 Series Software	Version 1.0.1 (for use with *Sentosa*® SA201)
Sentosa® SA201 Reporter	Version 1.5 (for use with *Sentosa*® SA201)

Sentosa® SX101 workflow

NOTE: Refer to *"Sample preparation (for specimen pooling)"* for steps to combine specimens into a pool.

Sample preparation (for individual samples)

Label empty 1.5 mL Safe-Lock tubes with NC, PC and sample IDs. Up to 46 samples, 1 positive control sample (PC) and 1 negative control (NC) sample can be performed in one run of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48).

Fresh samples

- Vortex swab in Universal Transport Media (UTM) for 30 seconds
- Remove and discard the swab from the UTM tube according to local safety regulations
- Transfer 230 µL of samples to 1.5 mL Safe-Lock Tubes

Samples stored at 4°C

- Equilibrate to room temperature
- Vortex swab in UTM for 30 seconds
- Remove and discard the swab from the UTM tube according to local safety regulations
- Transfer 230 µL of samples to 1.5 mL Safe-Lock Tubes

Frozen samples

- Thaw samples and equilibrate to room temperature
- Vortex swab in UTM for 30 seconds
- Remove and discard the swab from the UTM tube according to local safety regulations
- Transfer 230 µL of samples to 1.5 mL Safe-Lock Tubes

1. Sample pre-treatment and lysis

1.1. Prepare the reagents from the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) according to steps 1.2 and 1.3.

1.2. Reconstitute cRNA (lyophilized carrier RNA) with Buffer D4 as described below.

- Briefly spin down cRNA for 5 seconds.
- Add 310 µL of Buffer D4 to cRNA tube.
- The reconstituted cRNA should be mixed by pulse vortexing for 30 seconds followed by brief centrifugation.
- Aliquot reconstituted cRNA into 4 tubes of 78 µL and store at -20°C till required.

1.3. Thaw NC, PC and EC8 completely, vortex for 5 seconds and centrifuge briefly.

1.4. Prepare the lysis master mix according to the table below.

Reagents	48 samples
Buffer D1	15 mL
Reconstituted cRNA	75 µL
EC8	500 µL

1.5. Pre-heat the thermomixer to 56°C.

1.6. Transfer 311 µL of lysis master mix into labeled 1.5 mL Safe-Lock microtubes (sample, NC and PC).

1.7. Pulse vortex NC and PC for 10 seconds, followed by brief centrifugation to collect the contents at the bottom of the tubes. Transfer 230 µL of NC, PC, and samples into individually labeled 1.5 mL Safe-Lock microtubes containing the lysis master mix (sample, NC and PC).

NOTE: Do not discard the original NC and PC tubes, as they will be required for barcode scanning in step 2.15.

1.8. Pulse vortex the 1.5 mL Safe-Lock microtubes for 10 seconds followed by brief centrifugation to collect the contents at the bottom.

1.9. Incubate the tubes at 56°C for 8 minutes under 1000 rpm agitation using a thermomixer.

1.10. Centrifuge the samples briefly to collect the contents at the bottom of the tubes and cool for 3 minutes at room temperature.

1.11. The sample tubes are ready to be loaded onto the *Sentosa*® SX101 instrument. Carefully load the sample tubes onto the *Sentosa*® SX Rack 0.5 + Adaptor / 1.5 / 2.0 mL in the *Sentosa*® SX101 according to the procedure described in the “Automated nucleic acid extraction and RT-PCR set up on the *Sentosa*® SX101 instrument” section, page 46.

2. *Automated nucleic acid extraction and RT-PCR set up on the Sentosa® SX101 instrument*

Figure 1 shows the positions of consumables / labware on the *Sentosa*® SX101 platform. Double line the *Sentosa*® SX Waste Bin with biohazard bags. Please refer to the layout as indicated by the *Sentosa*® SX101 instrument software or the appendix to load all items in the correct positions.

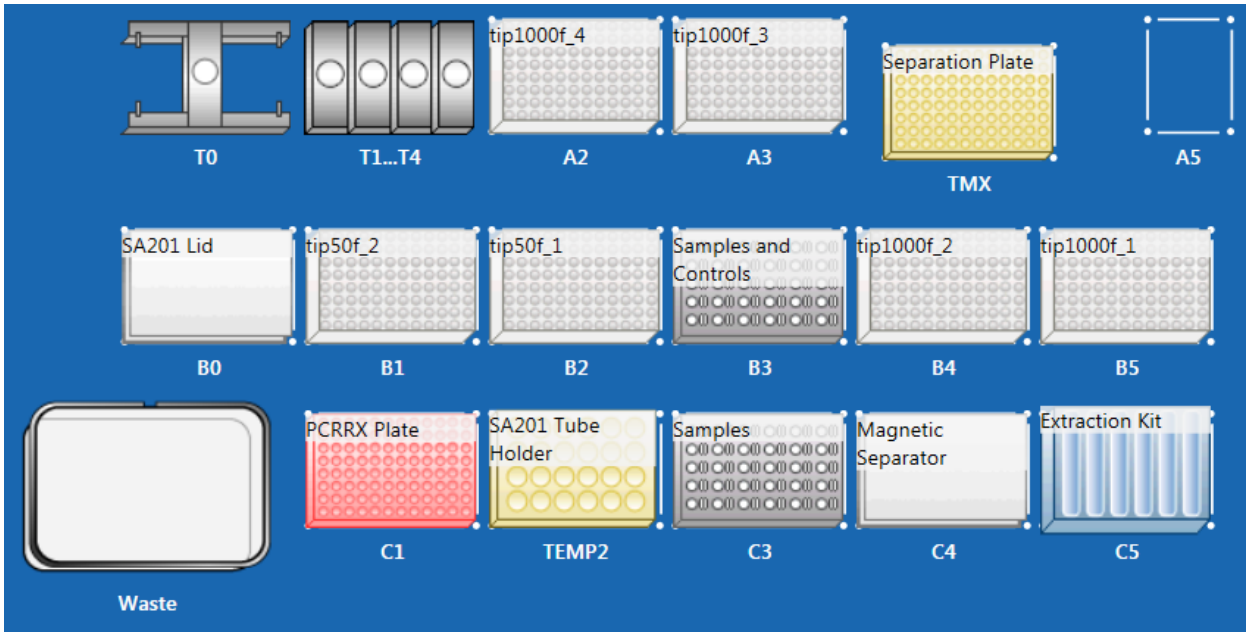


Figure 1. Layout of the *Sentosa*® SX101 platform for 96 tests

NOTE:

- Items shown are necessary for nucleic acid extraction and PCR assay set-up for application “48-1 ViroKey SARS-CoV-2-v2 v3-3”.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the *Sentosa*® SX Waste Bin before starting a protocol run. For more information, please refer to the *Sentosa*® SX101 instrument user manual.

2.1. Switch on the computer, and wait for the initialization procedure to be completed.

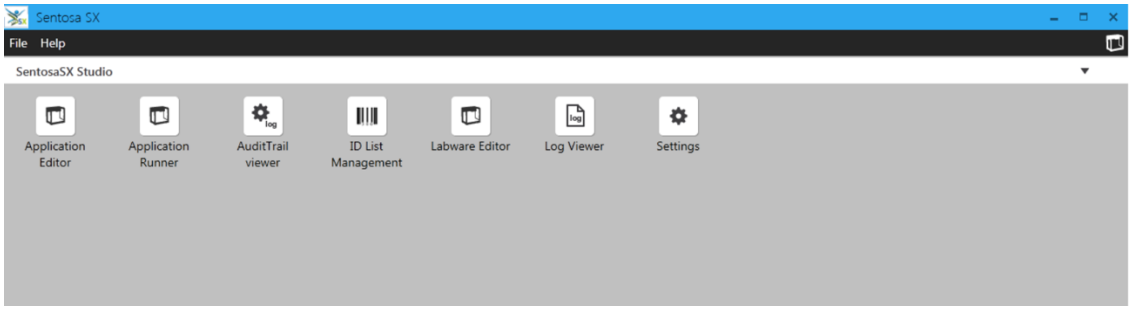
2.2. On the instrument's computer, launch the *Sentosa*® SX software by double-clicking the  icon.

The *"Login"* window opens.

Type the account name and password, and then click *"Login"*.

NOTE: Please switch on *Sentosa*® SX101 instrument after *Sentosa*® SX software is launched.

2.3. Press / Click on *"Applications Runner"* in the start screen menu to select and open an application.



2.4. Select the *"VelaDx"* account and choose the folder *"Sentosa SX Virus"*. Run the suitable application according to the table below.

Application (v3-3 or higher)	Number of tests	ViroKey® SX Virus Total Nucleic Acid Kit (4x48)	ViroKey® SARS-CoV-2 RT-PCR Test v2.0
48-1 ViroKey SARS-CoV-2-v2 v3-3	Up to 46 tests	1 set of reagents	1 set of reagents

2.5. Place the correct *Sentosa*® SX Reservoirs and Modules into positions 1 to 7 of the *Sentosa*® SX Reservoir Rack 7 (refer to **Figure 1**, C5) according to the table below.

Application (v3-3 or higher)	*Sentosa*® SX Reservoir Rack 7 (C5)			
	Position 1 & 5	**Positions 2 to 4**	**Position 6**	**Position 7**
48-1 ViroKey SARS-CoV-2-v2 v3-3	Empty	3 x 100 mL Reservoir	1 x 30 mL Reservoir	RR Module A1/A2

2.6. Add 40 mL of absolute ethanol to Buffer D3 and mix well before use.

2.7. Transfer all buffers from the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) into their respective positions in *Sentosa*® SX Reservoir Rack 7 as indicated by the application protocol: 17 mL of D1 in positions 2, 35 mL of D2 in position 3, 50 mL of D3 in position 4 and 4 mL of D4 in position 6.

Application (v3-3 or higher)	*Sentosa*® SX Reservoir Rack 7 (C5)					
	Position 1	**Position 2**	**Position 3**	**Position 4**	**Position 5**	**Position 6**
48-1 ViroKey SARS-CoV-2-v2 v3-3	Empty	D1	D2	D3	Empty	D4

NOTE:

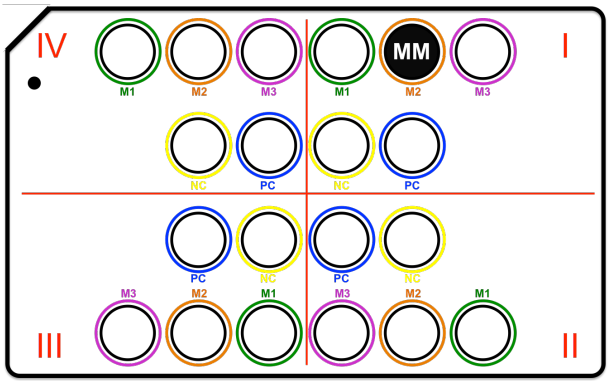
- 17 mL of Buffer D1 should be added to position 2, excess buffer may change the beads to sample ratio which may affect its performance for samples at the LoD level.
- All reagents should be gently mixed, without foaming, before use.
- Ensure that all buffers in the bottles are completely transferred into the corresponding reservoir positions.

IMPORTANT: Correct placement of assay components is critical for successful completion of the test. Please follow on-screen prompt carefully for correct placement of components.

2.8. Prepare Reverse Transcription PCR reagents from the ViroKey® SARS-CoV-2 RT-PCR Test v2.0. Pulse vortex SARS-CoV-2 v2 M1 (M1) for 5 seconds, and mix RNA4 M2 (M2) and RNA4 M3 (M3) by gentle inversion. Keep the M3 tube on ice for no more than one hour during use.

Pipette 112 µL of M3, 56 µL of M1 into separate tube of M2. Centrifuge reconstituted M1/M2/M3 (MM) briefly to collect the contents at the bottom of the tubes.

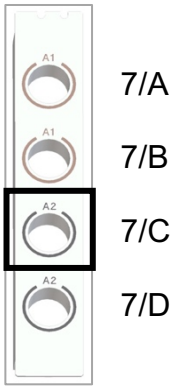
Remove the caps of the MM tube (reconstituted M1 / M2 / M3), and load the MM tube into the SA201 Tube Holder rack (Location: TEMP2, C2) at M2 position in Quadrant I.



NOTE:

- Do not discard the original M1 tube, as it will be required for barcode scanning in step 2.17.
- Do **not** mix components from kits with different lot numbers.

2.9. Place the *Sentosa*® SX RR Module A1/A2, illustrated in the figure below, on position 7 of the *Sentosa*® SX Reservoir Rack 7.



Sentosa® SX RR Module A1/A2

Load Mag (magnetic beads) tube from the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) on the *Sentosa*® SX RR Module A1/A2 according to the table below.

Application (v3-3 or higher)	*Sentosa*® SX Reservoir Rack 7 (C5)			
	7/A	7/B	7/C	7/D
48-1 ViroKey SARS-CoV-2-v2 v3-3	Empty	Empty	Mag	Empty

NOTE:

- Ensure that the magnetic beads in Mag tube from ViroKey® SX Virus Total Nucleic Acid Kit (4x48) are fully re-suspended. Vortex the tubes for 1 minute and flick to collect the contents at the bottom of the tube. Ensure that no bubbles or multilayers are observed.
- Ensure that all tubes are uncapped.

IMPORTANT: Correct placement of assay components is critical for successful completion of the test. Please follow on-screen prompt carefully for correct placement of components.

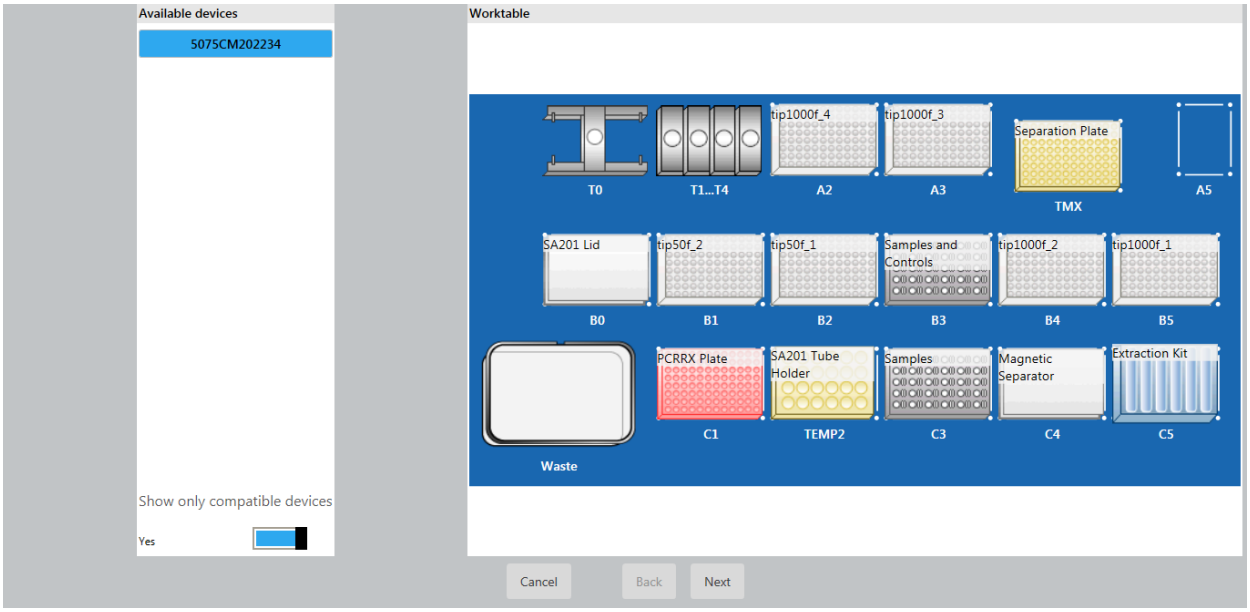
2.10. Set the MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL on the epMotion® Thermoblock for PCR 96 Wells Thermoblock PCR 96, and place the assembly on position C1 of the *Sentosa*® SX101 platform (refer to **Figure 1**).

MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL

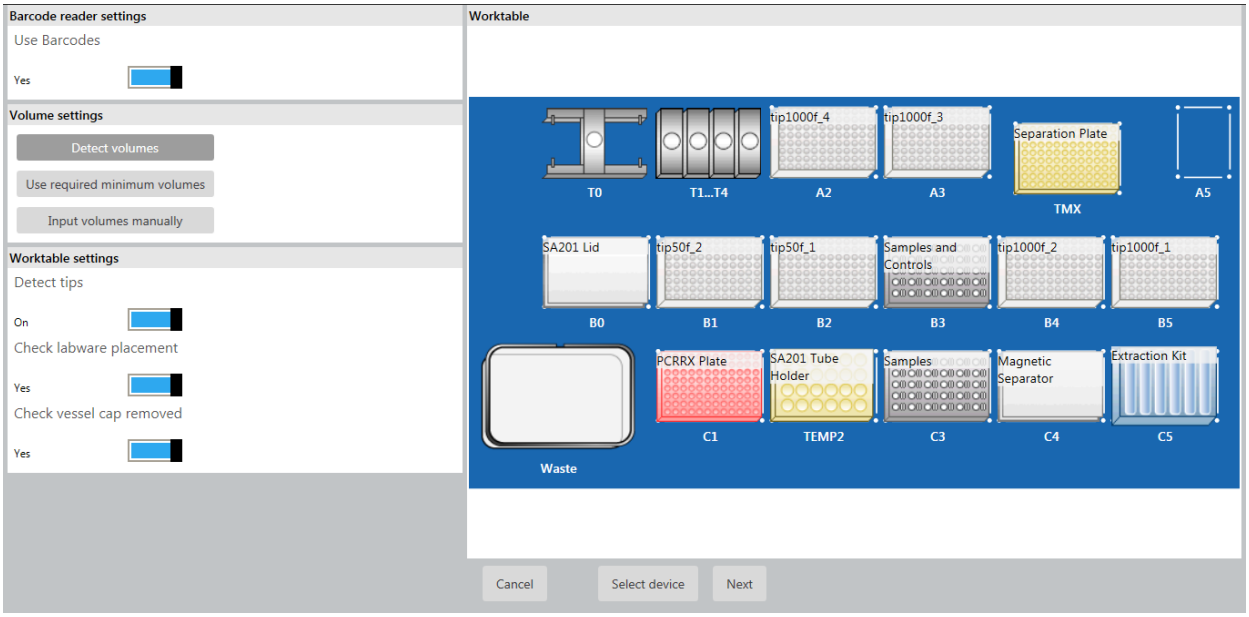
epMotion® Thermoblock for PCR 96 Wells

2.11. Load the *Sentosa*® SX Non-Partition 1000 µL Filter Tips, *Sentosa*® SX Deepwell Plate 96/2000 µL, *Sentosa*® SX Non-Partition 50 µL Filter Tips and *Sentosa*® SX Magnetic Separator on the *Sentosa*® SX101 platform. Please refer to the layout as indicated by the *Sentosa*® SX101 instrument software (see **Figure 1**) or the appendix to load all items in the correct positions.

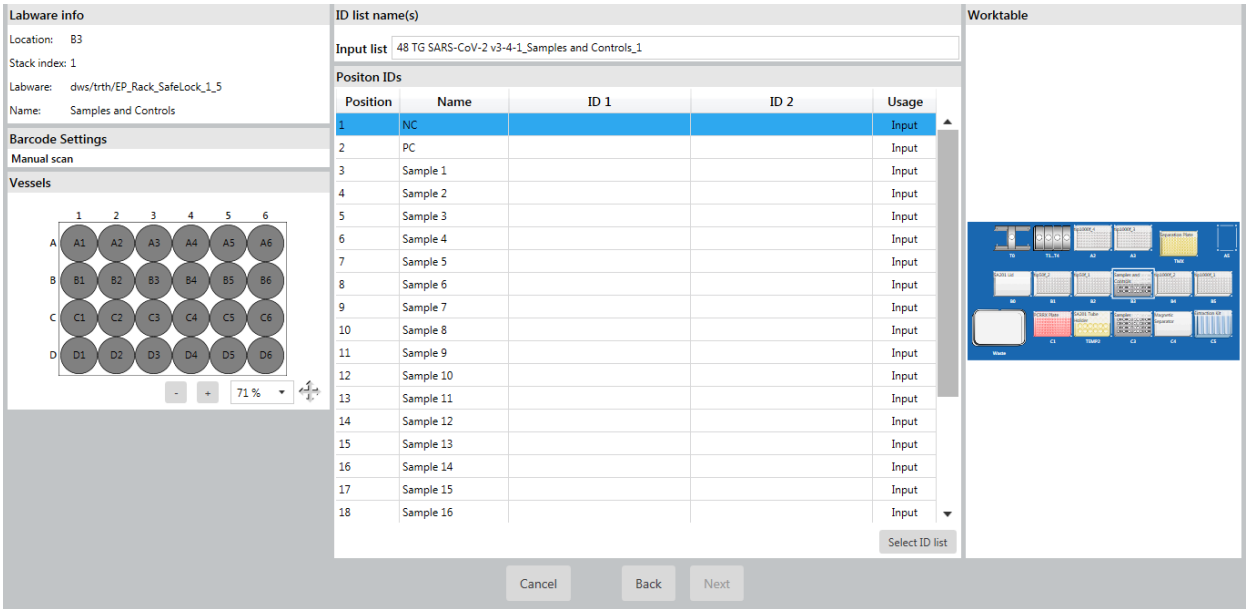
2.12. In the *"Run"* tab, select the *Sentosa*® SX101 instrument (5075XXXXX, where XXXXX refers to the serial number) under *"Available devices"* is selected with the "*Yes*" slider appearing blue. Press / Click "*Next*.



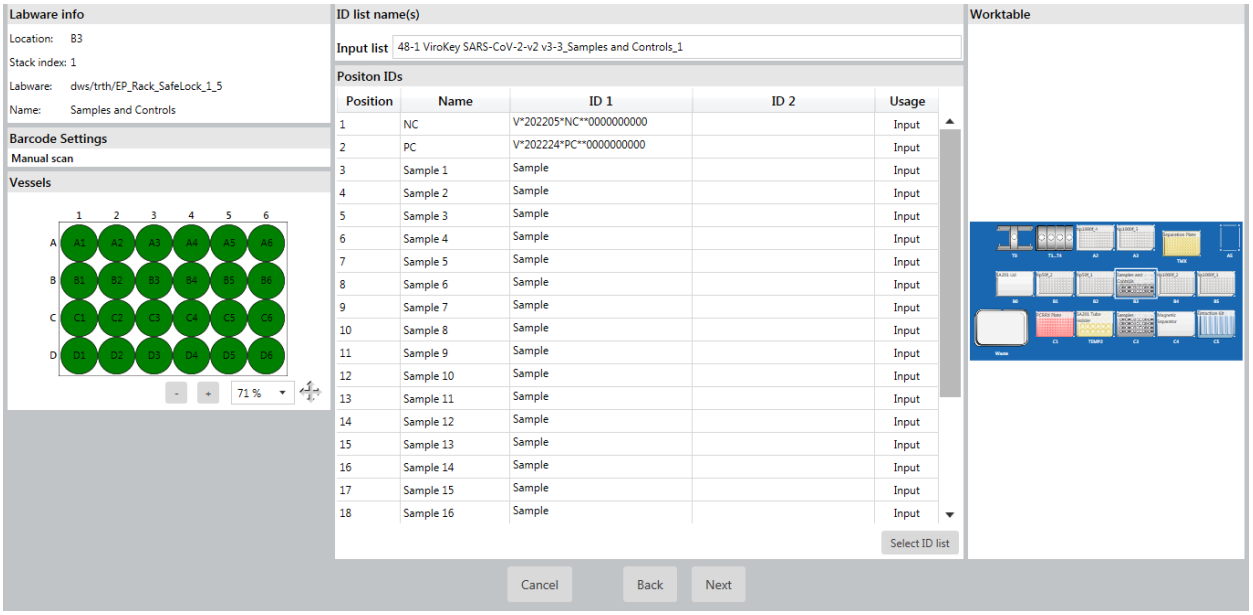
2.13. Ensure that all options are turned on: all sliders are blue and annotated as "*Yes*" or "*On*" under all settings as shown in the following figure. Press / Click *"Next"*.



2.14. Enter the run name manually by checking the "*Enter manually*" check box. Click "*OK*" and then "*Next*".



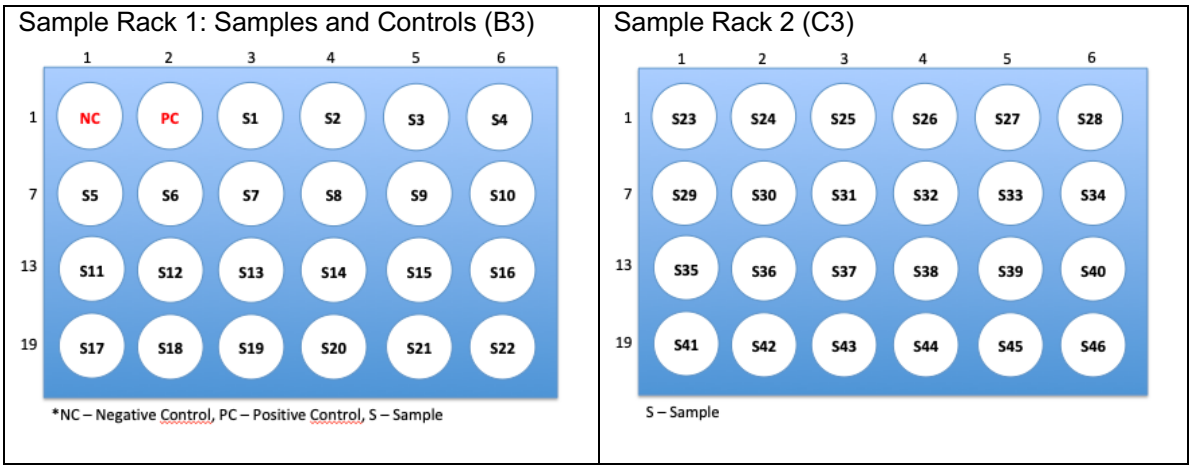
2.15. Scan the NC, PC and samples' ID barcodes according to the table below. If the samples do not have ID barcodes, enter the sample IDs manually by checking the *"Enter manually"* check box.



Application (v3-3 or higher)	*Sentosa*® SX Rack 0.5 + Adaptor / 1.5 / 2.0 mL		
	Sample rack (B3)		
	Position 1	**Position 2**	**Position 3 – 24**
48-1 ViroKey SARS-CoV-2-v2 v3-3	NC	PC	Samples

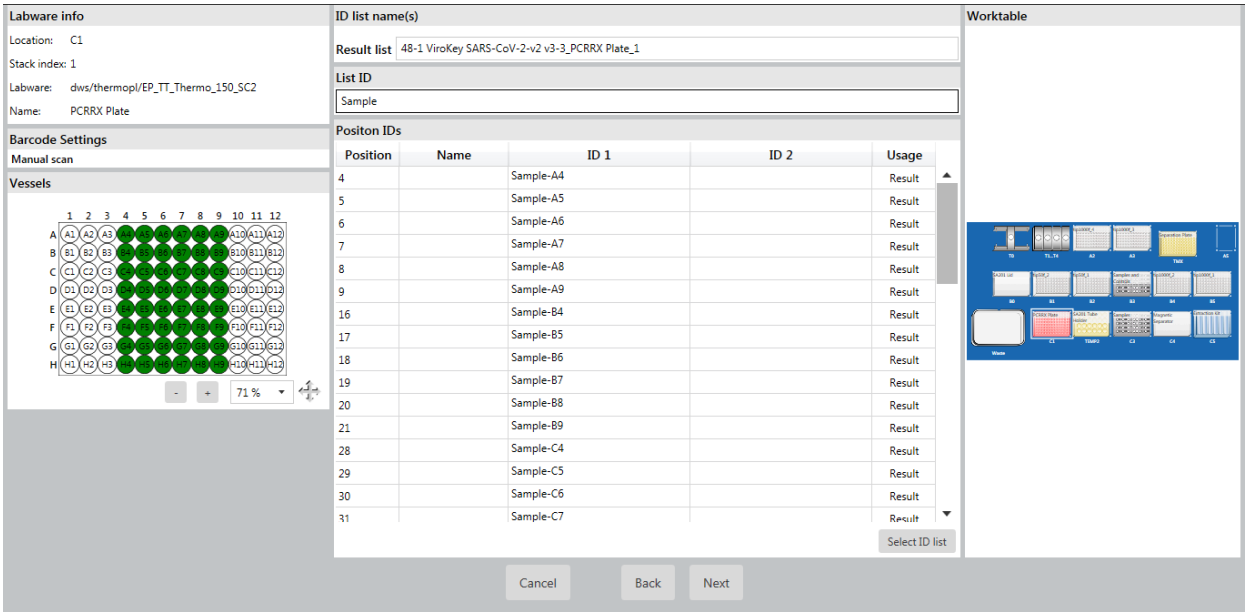
Once all samples are identified, click "*OK*" and then "*Next*".

2.16. Load NC, PC and samples onto the *Sentosa*® SX Rack 0.5 + Adaptor / 1.5 / 2.0 mL (refer to Figure 1, B3 & C3) as illustrated below.



NC – Negative control, PC – Positive control, S – Sample

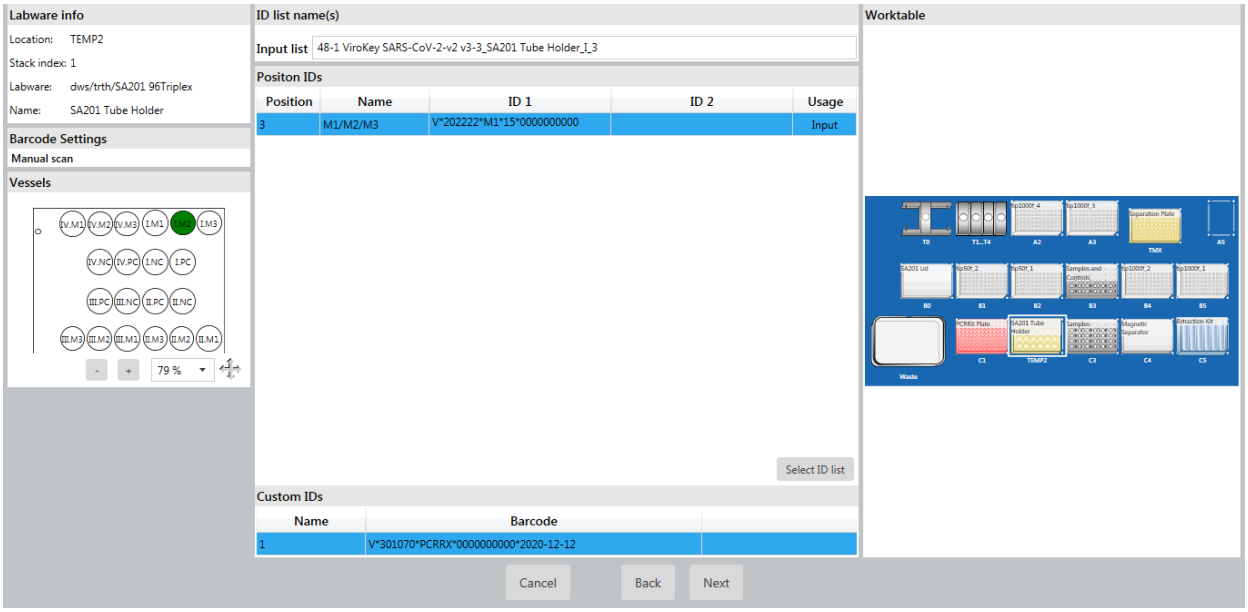
2.17. Scan the barcode on the *Sentosa*® SX Barcoded PCR Plate 96 (reaction plate) or enter the ID manually under "*List ID*". Press / Click *"Next"*.



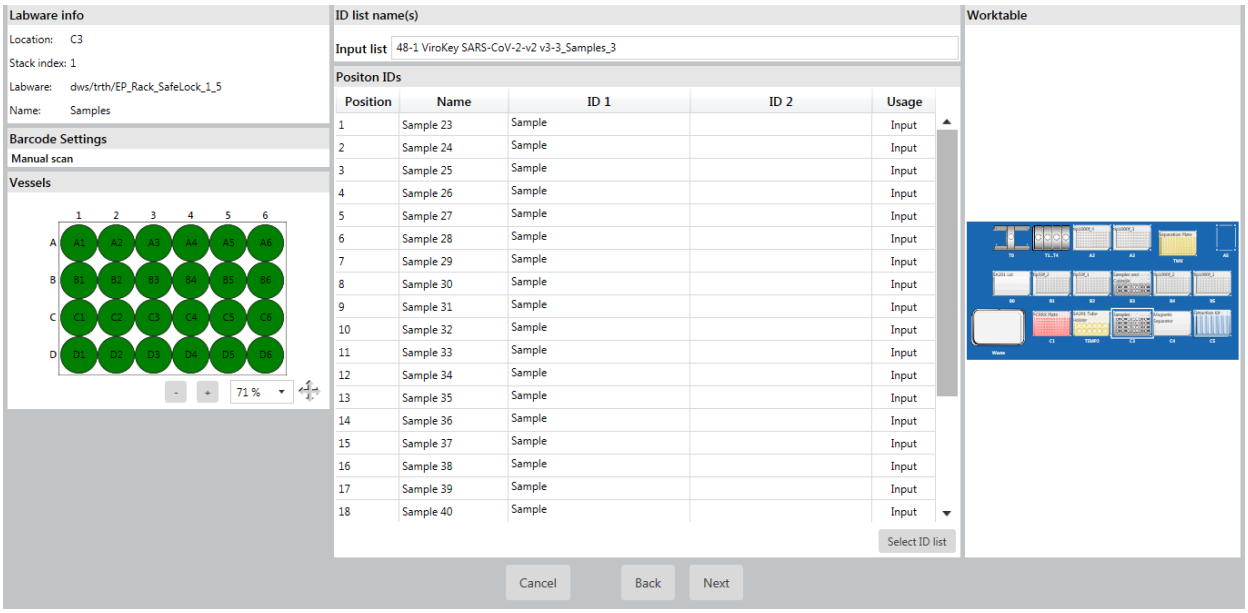
After scanning, place the reaction plate back onto the *Sentosa*® SX Thermoblock PCR 96 OC (refer to **Figure 1**, Location: C1). The reaction plate should be oriented with the barcode label facing towards the user.

IMPORTANT: Correct placement of assay components is critical for successful completion of the test. Please follow on-screen prompt carefully for correct placement of components.

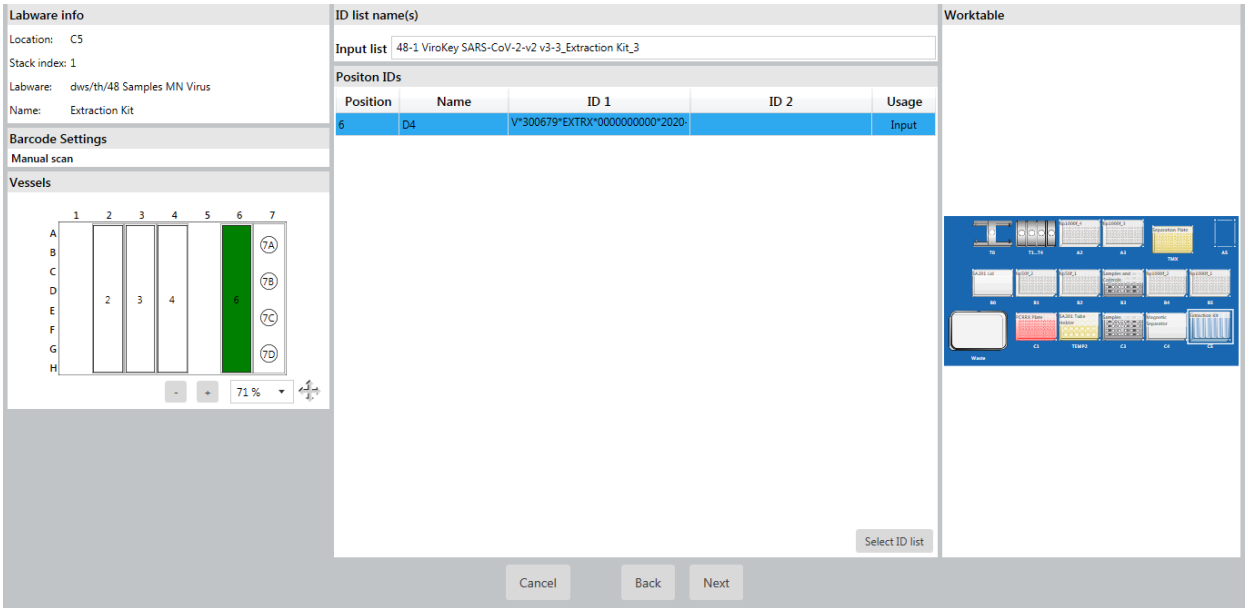
2.18. For the reconstituted M1 / M2 / M3 mixture stored in the M2 tube (MM), scan the empty M1 tube ID barcode to ensure the correct test kit is used. Click *"OK"* and then *"Next"*.



2.19. Scan samples' ID barcodes according to the table below for the last 24 samples. If the samples do not have ID barcodes, enter the sample IDs manually by checking the "*Enter manually*" check box.



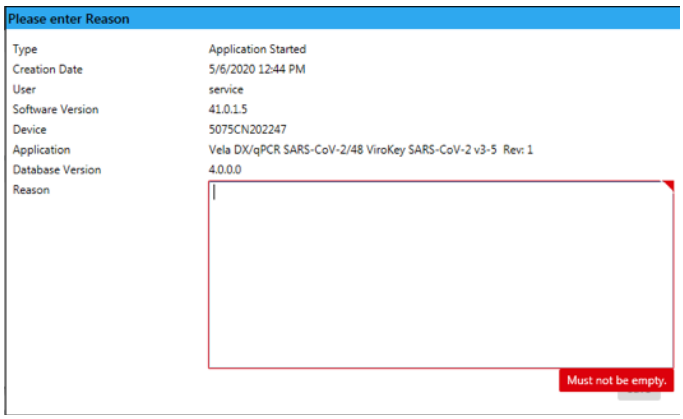
2.20. Scan the 2-D barcode on the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) box that will be used in the run. Press / Click *"Next"*.



2.21. The software conducts a volume check for each item; click *"Next"* for each "*volume check*" window.

- *Sentosa*® SX Deepwell Plate 96/2000 µL (Location: TMX)
- *Sentosa*® SX Rack 0.5 + Adaptor / 1.5 / 2.0 mL (Location: B3 and C3)
- SA201 Tube Holder Rack (Location: TEMP C2)
- *Sentosa*® SX Reservoir Rack 7 (Location: C5)

At the last window, press / click *"Run"*.

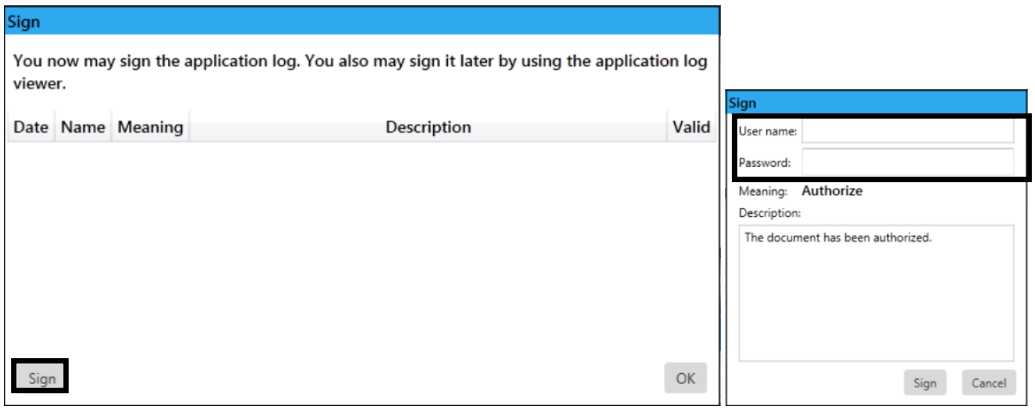


In the *"Please enter Reason"* pop-up window, enter a reason for the run in the textbox if applicable, if not, enter any alphanumeric character / symbol key and press / click *"Save"* to continue.

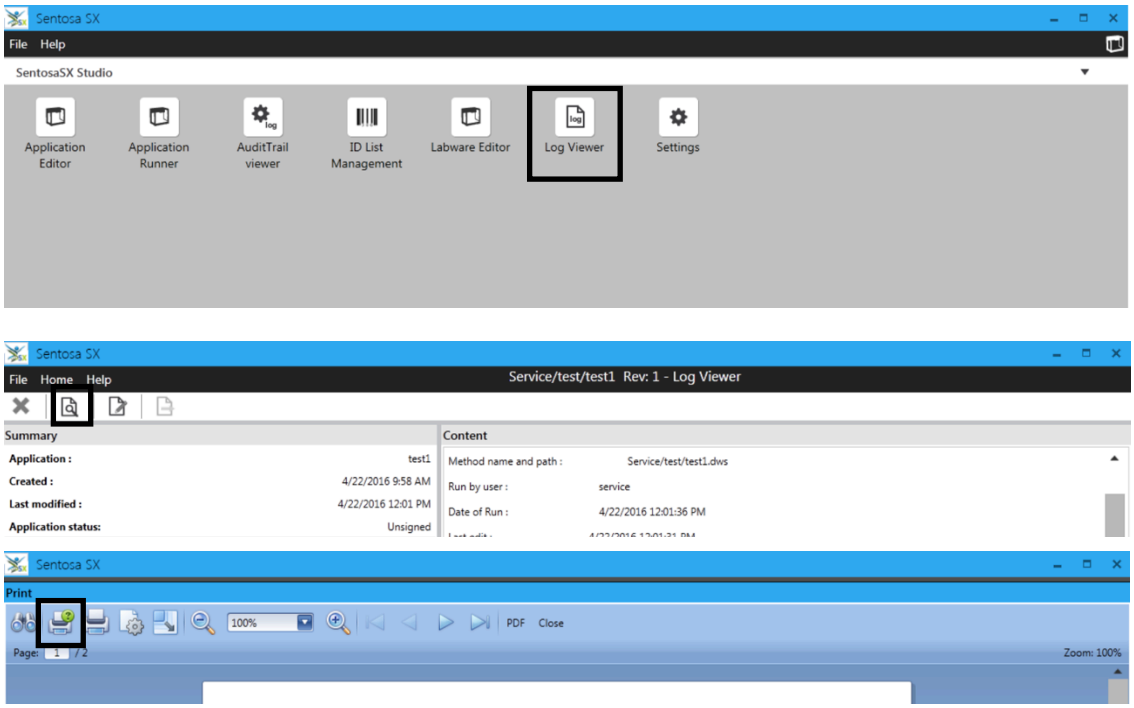
2.22. Nucleic acid extraction and PCR set-up steps are performed automatically. At the end of the protocol, the status of the run changes from *"running"* to *"completed"*.
After the run is completed, carefully remove the MicroAmp® Fast Optical 96-Well Reaction Plate. Apply the MicroAmp® Optical Adhesive Film over the plate and seal the plate tightly to prevent contamination. Briefly spin down the PCR plate and load it onto the *Sentosa*® SA201 Real-Time PCR Instrument or the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument. Proceed to RT-PCR and data analysis using the *Sentosa*® SA201 Real-Time PCR Instrument and *Sentosa*® SA201 Reporter (pages 91 to 105) or Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with the ABI SDS Software for manual data analysis (pages 106 to 119).

After RT-PCR and data analysis are complete, proceed to page 12 for *"Result interpretation"*.

2.23. Log file of the run is automatically generated which is then electronically signed by the operator by clicking *"Sign"*.



2.24. To print the log file, press / click on *"Log Viewer"* in the start screen, select the respective log and press / click the icon followed by the icon.



2.25. Clean and disinfect the *Sentosa*® SX101 instrument after each run. For instrument maintenance, please refer to the *Sentosa*® SX101 instrument user manual.

KingFisher workflow

3. Buffer, Reagent and Sample Preparation

3.1. Buffer and Reagent preparation:

Remove the following reagents from the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) and prepare according to the instructions.

Storage	Reagents	Quantity	Preparation
RT	D1 (Lysis buffer)	2	Gently invert to mix.
	D2 (Wash buffer 1)	2	Mix by gentle inversion, briefly spin down.
	D3 (Wash buffer 2)	2	Add 40 mL molecular grade absolute ethanol to D3 bottle. Mix well.
	D4 (ddH_2O)	2	• Briefly spin down. Reconstitute cRNA with 310 µL of D4. • Vortex and spin down.
	cRNA (lyophilized carrier RNA)	2	

			• Aliquot reconstituted cRNA into 4 tubes of 78 µL. • Store cRNA at -20°C until use.
	Magnetic Beads	2	Vortex for 5 minutes to fully resuspend magnetic beads.

Remove the following reagents from the ViroKey® SARS-CoV-2 RT-PCR Test Kit v2.0 and prepare according to the instructions.

Storage	Reagent	Quantity	Preparation
-20°C	NC	1	Thaw completely, vortex for 5 seconds and spin down
	PC	1	
	EC8	2	

Prepare Lysis master mix with the following volumes.

Reagents	96 samples
Buffer D1	34.0 mL
Reconstituted cRNA	156 µL
EC8	1.2 mL

NOTE:
- All reagents should **not** be subjected to more than five repeated freeze-thaw cycles.

3.2. Sample preparation (for individual patient samples)

NOTE: Refer to *"Sample preparation (for specimen pooling)"* for steps to combine specimens into a pool.

Fresh samples
- Vortex swab in Universal Transport Media (UTM) for 30 seconds.
- Remove and discard the swab from the UTM tube according to local safety regulations.
- Transfer 230 µL of samples to the KingFisher™ 96-Deepwell Plate (DWP) according to **Figure 2.**

Samples stored at 4 °C
- Equilibrate to room temperature.
- Vortex swab in UTM for 30 seconds.

- Remove and discard the swab from the UTM tube according to local safety regulations.
- Transfer 230 µL of the sample to the DWP according to **Figure 2.**

***Frozen samples**:*

- Thaw samples and equilibrate to room temperature.
- Vortex swab in UTM for 30 seconds.
- Remove and discard the swab from the UTM tube according to local safety regulations.
- Transfer 230 µL of the sample to the DWP according to **Figure 2.**

3.3. Transfer of samples to the Deepwell Plate—for both individual patient samples & pooled samples

3.3.1. Add 230 µL of NC and PC to well A1 and B1 in the DWP.

3.3.2. Dispense 309 µL of Lysis master mix (LMM) into each well of the Sample Deep Well Plate to inactivate the virus.

3.3.3. Add samples according to **Figure 2.** below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
B	PC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
C	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89

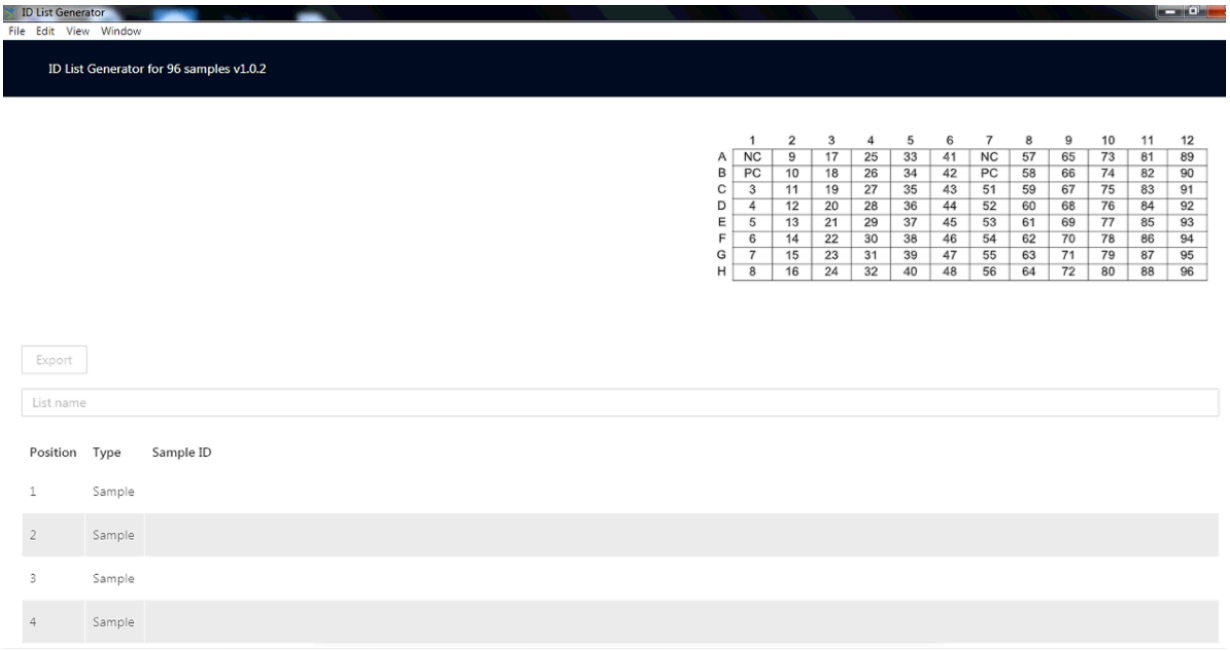
D	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
E	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91
F	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84	S92
G	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85	S93
H	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86	S94

Figure 2. Layout of samples in the KingFisher™ 96-Deepwell Plate.

3.4. Set up ID List of samples on the *Sentosa*® SX101

3.4.1. Start the *"ID List Generator"* software and log in to your account.

3.4.2. Scan NC barcodes in position 1 and PC barcodes in position 2, followed by Sample Name of each sample.



	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	9	17	25	33	41	NC	57	65	73	81	89
B	PC	10	18	26	34	42	PC	58	66	74	82	90
C	3	11	19	27	35	43	51	59	67	75	83	91
D	4	12	20	28	36	44	52	60	68	76	84	92
E	5	13	21	29	37	45	53	61	69	77	85	93
F	6	14	22	30	38	46	54	62	70	78	86	94
G	7	15	23	31	39	47	55	63	71	79	87	95
H	8	16	24	32	40	48	56	64	72	80	88	96

3.4.3. Export Sample ID list (e.g. SampleList.xml) for import into the *Sentosa*® SX101.

4. Automated RT-PCR set up on the Sentosa® SX101 instrument

Figure 3 shows the positions of consumables / labware on the *Sentosa*® SX101 platform. Double line the *Sentosa*® SX Waste Bin with biohazard bags. Please refer to the layout as indicated by the *Sentosa*® SX101 instrument software or the appendix to load all items in the correct positions.

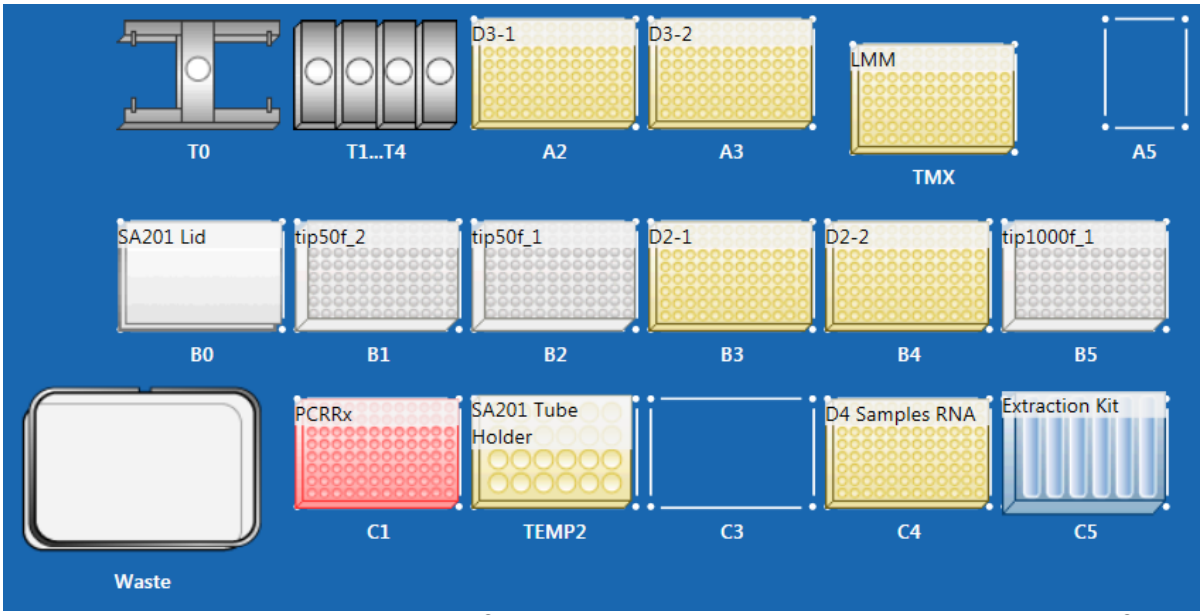


Figure 3. Layout of the *Sentosa*® SX101 platform for 96 tests with ViroKey® SX Virus Total Nucleic Acid Kit (4x48).

NOTE:

- Items shown are necessary for PCR assay set-up for application "96-1_ViroKey-KF SARS-CoV-2-v2_v3-2".
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the *Sentosa*® SX Waste Bin before starting a protocol run. For more information, please refer to the *Sentosa*® SX101 instrument user manual.

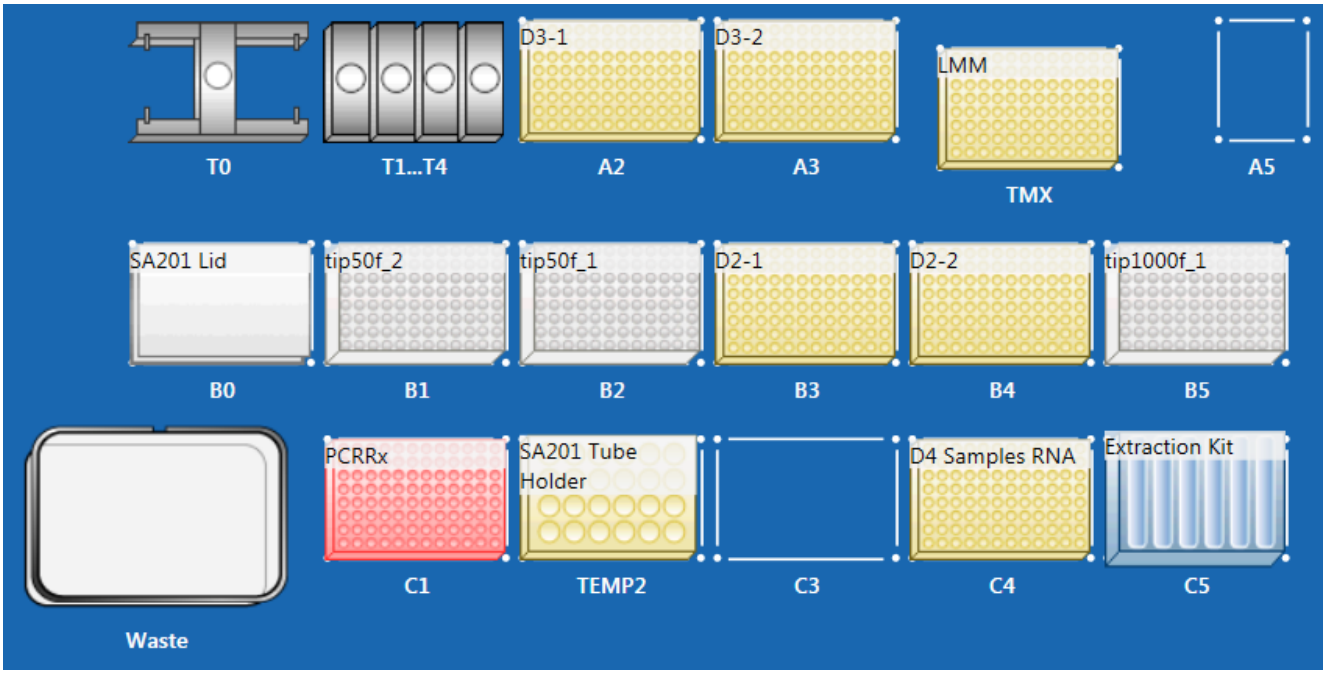
4.1. Start the Sentosa SX software and log in to your account.

4.2. Click on *"Application Runner"* on the start screen.

4.3. Perform the following steps:

- Select VelaDx Account
- Open ***"qPCR ViroKey"*** folder
- Launch ***"96-1_ViroKey-KF SARS-CoV-2-v2_v3-2"*** application

4.4. Prepare worktable according to the layout below.



4.5. Place the correct *Sentosa*® SX Reservoirs and Modules into positions 1 to 7 of the *Sentosa*® SX Reservoir Rack 7 (C5) according to the table below.

Application (v3-1 or higher)	***Sentosa*® SX Reservoir Rack 7 (C5)**			
	Position 1 – 3	**Position 5**	**Position 4 & 6**	**Position 7**
96-1_ViroKey-KF SARS-CoV-2-v2_v3-2	3 x 100 mL Reservoir	RR Module A1/A2	Empty	30 mL Reservoir

4.6. Add 40 mL of absolute ethanol to each of the 2 bottles Buffer D3 and mix well before use.

4.7. Transfer all buffers from the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) into their respective positions in *Sentosa*® SX Reservoir Rack 7 as indicated by the application protocol (refer
to the table below).

Buffer	**Position**	**Volume (mL)**

1x D1	1	35
2x D2	2	70
2x D3	3	100
2x D4	7	12

Application (v3-1 or higher)	***Sentosa*® SX Reservoir Rack 7 (C5)**					
	Position 1	**Position 2**	**Position 3**	**Position 4**	**Position 6**	**Position 7**
96-1_ViroKey-KF SARS-CoV-2-v2_v3-2	D1	2x D2	2x D3	Empty	Empty	2x D4

4.8. Place the *Sentosa*® SX RR Module A1/A2 on position 5 of the *Sentosa*® SX Reservoir Rack 7.

4.9. Load Mag (magnetic beads) tubes from the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) on the *Sentosa*® SX RR Module A1/A2 according to the table below.

Application (v3-1 or higher)	***Sentosa*® SX Reservoir Rack 7 (C5)**			
	5/A	**5/B**	**5/C**	**5/D**
96-1_ViroKey-KF SARS-CoV-2-v2_v3-2	Mag	Empty	Empty	Empty

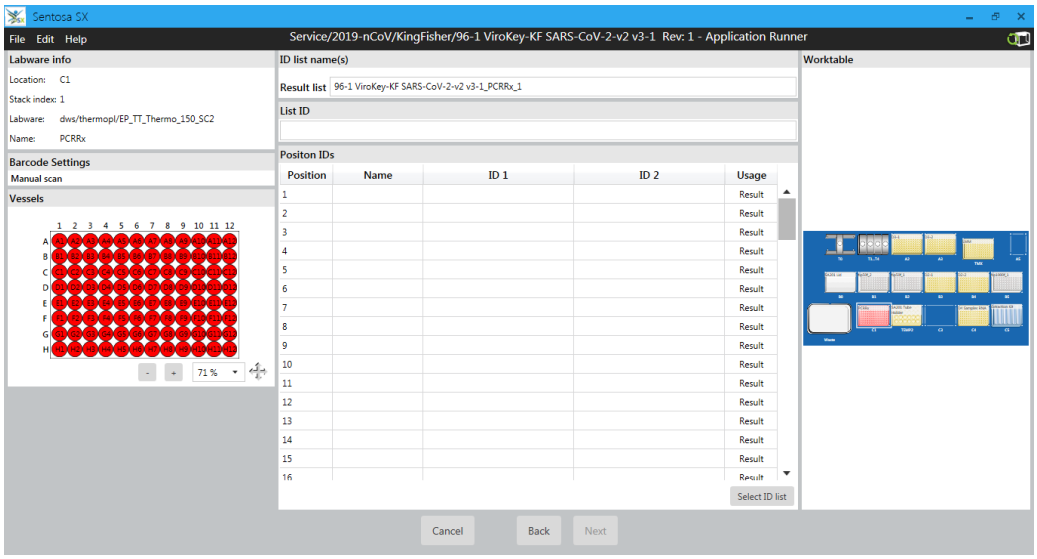
4.10. Start the run

4.10.1. Proceed to the *"Run"* tab when all the labware, consumables and reagents are in place. Click *"Next"*.

4.10.2. Make sure all of the levels checked are blue. Click *"Next"*.

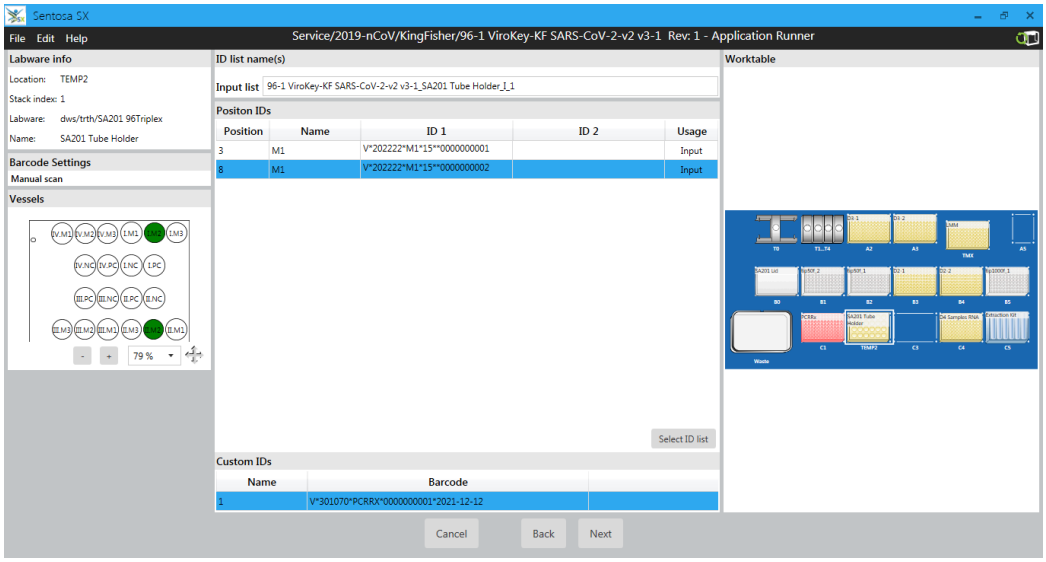
4.10.3. When prompted scan the barcodes of the following labware:

a. PCR plate (Position C1)—click *"Next"*.

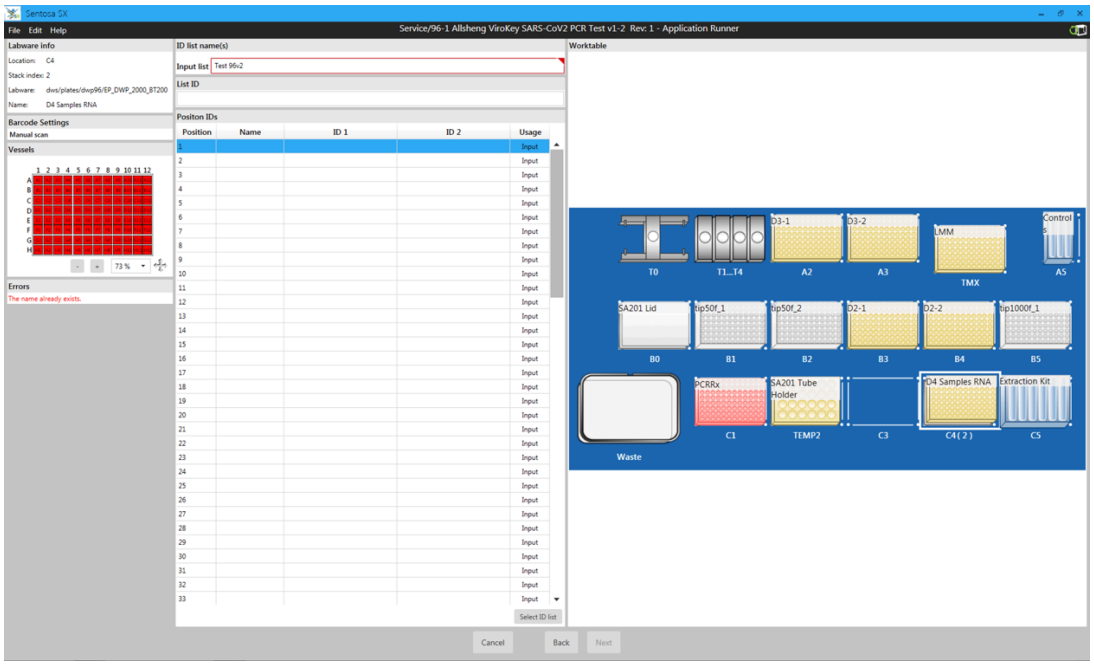


b. 2D barcodes of M1 tubes

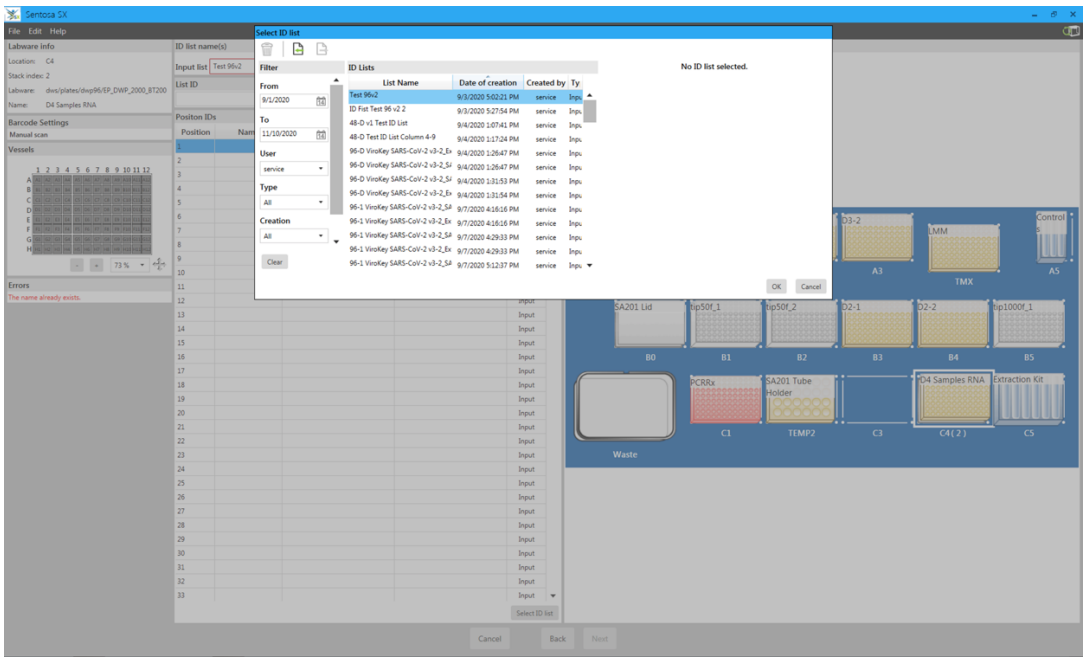
c. 2D barcode on the PCR kit box—click *"Next"*.



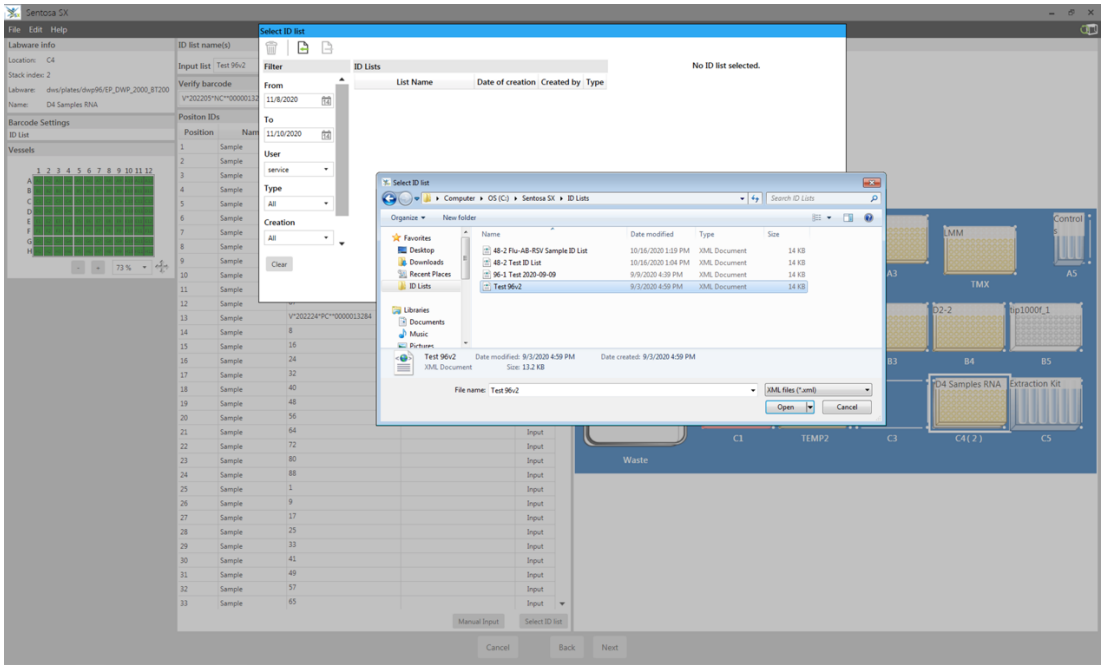
d. Click on *"Select ID List"*.



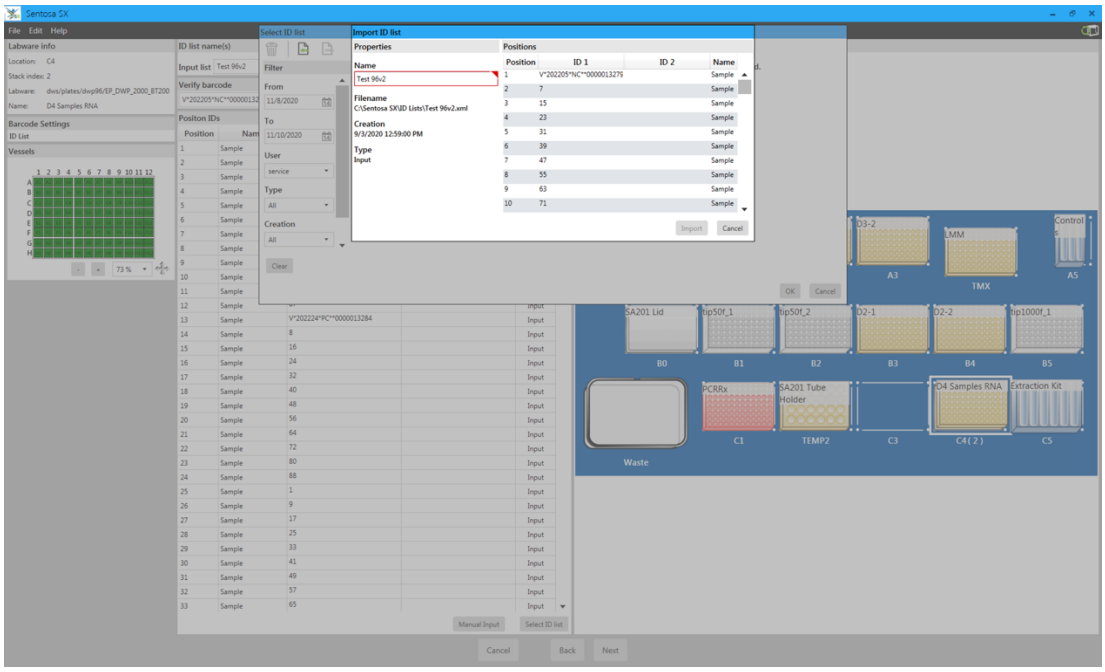
e. Click on import file icon.



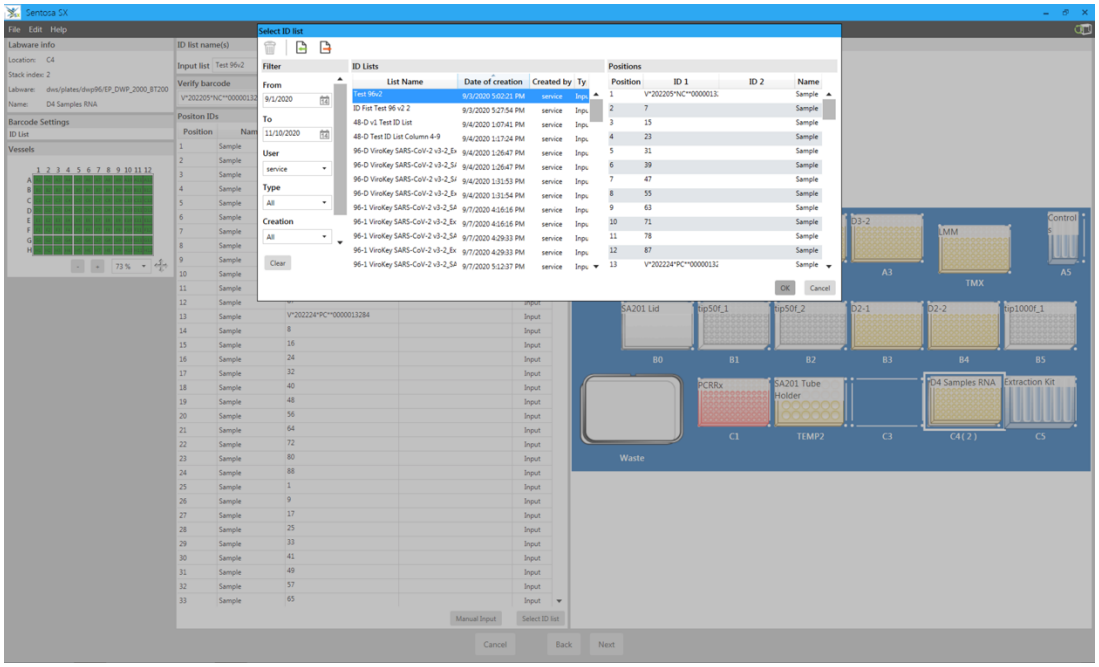
f. Select .xml File to import from folder.



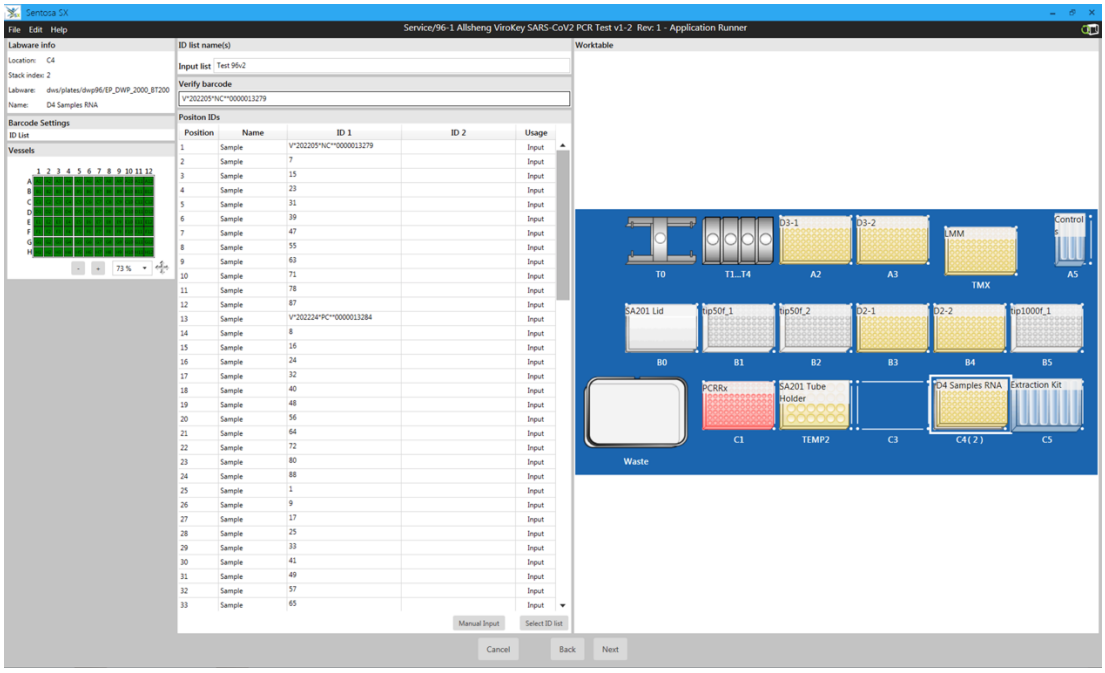
g. Click *"Import"*.



h. Select the ID list and click *"OK"*.



i. Select the ID list and click *"OK"*.

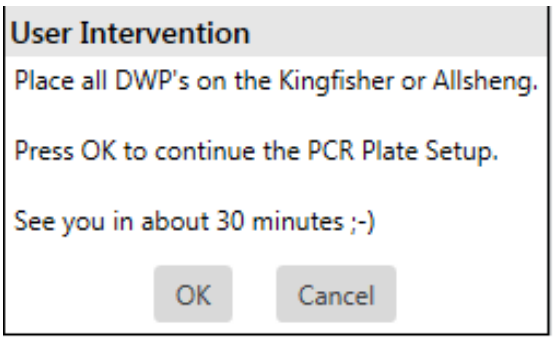


4.10.4. Click *"Next"* for all the volume-check windows.

4.11. User Interventions and Run completion

4.11.1. First User Intervention

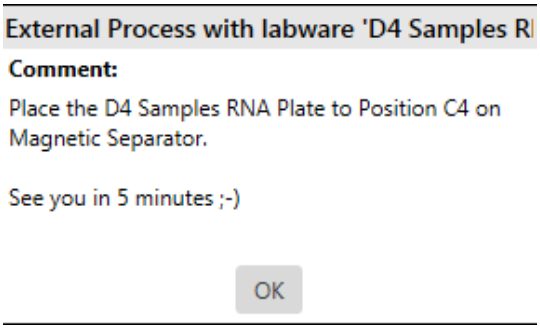
- At the first User Intervention window, open the *Sentosa*® SX101 and place all DWP's reagents and samples onto the KingFisher instrument. Then, run the KingFisher application *"96-1_ViroKey_VTNA_v1-1"*.



- Click *"OK"* to begin the extraction on the KingFisher instrument. Once the extraction has completed, proceed to click *"OK"* on the *Sentosa*® SX101 to initiate the transfer of the PCR MM into the PCR Plate.

4.11.2. Second User Intervention

- At the second User Intervention window, remove the D4-Samples plate from the KingFisher instrument and place onto the position C4 on the *Sentosa*® SX101.



- Click *"OK"* to begin the sample transfer to the PCR plate on *Sentosa*® SX101.
- At the end of the run, click *"Exit"*. A .smp file will be generated in the following directory *"C:\Sentosa SX\SMP"*.
- Save the .smp file in a thumb drive.
- Seal the PCR plate accordingly with Optical adhesive Seal and briefly spin down.

  NOTE: Ensure the plate is well sealed.

Hamilton workflow

Sample preparation (for individual samples)

NOTE: Refer to *"Sample preparation (for specimen pooling)"* for steps to combine specimens into a pool.

Sample in Universal Transport Medium (UTM) or Viral Transport Medium (VTM) tubes **must** be heat-inactivated at 75°C for 30 minutes before placing onto the Hamilton Microlab® STAR™ instrument with the cap and swab removed for sample transfer[7]. Up to 376 samples, 4 positive control sample (PC) and 4 negative control (NC) sample can be performed in one run of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) resulting in 4 PCR plates.

Fresh samples

- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

Samples stored at 4°C

- Equilibrate to room temperature.
- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

Frozen samples

- Thaw samples and equilibrate to room temperature.
- Vortex swab in Universal Transport Media for 30 seconds.
- Remove swabs from the tubes and discard the swabs according to the local safety regulations.
- Inactivate the SARS-CoV-2 virus in samples in an oven / a water bath at 75°C for 30 minutes.

Sample Tube Criteria for Hamilton Microlab® STAR™ instrument

<table>
<tr><th>Sample Tube Criteria</th><th>Description</th></tr>
<tr><td>Specifications</td><td>

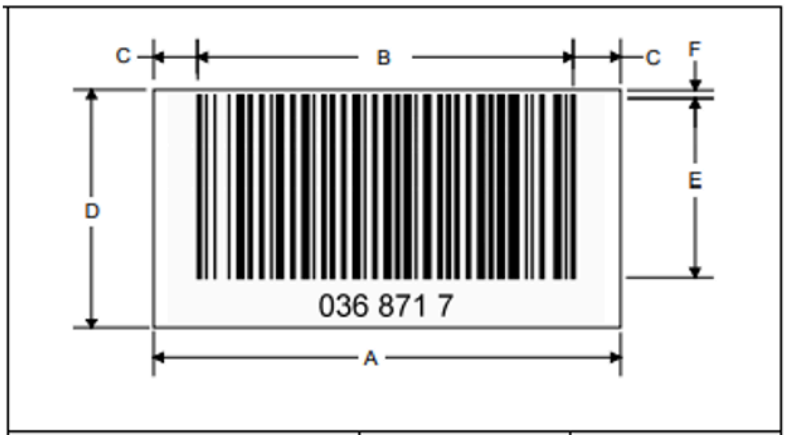
- **Diameter:** Between 14.5mm to 18mm
- **Height:** Between 60mm to 120mm

</td></tr>
<tr><td>Sample tube ID (Barcode)</td><td>

- **Maximum** of 20 characters
- **Only** alphanumeric characters are allowed (i.e. no symbols are allowed) for sample tube ID barcodes.
- Symbols are allowed for ID barcodes for kits, NC and PC.

</td></tr>
<tr><td>Barcode labels</td><td>

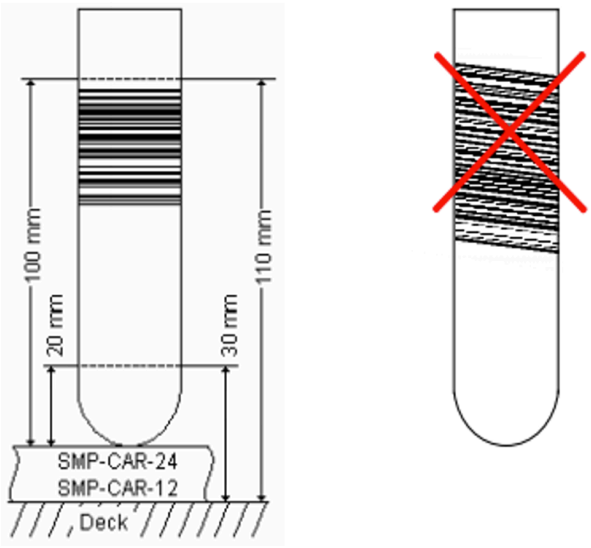
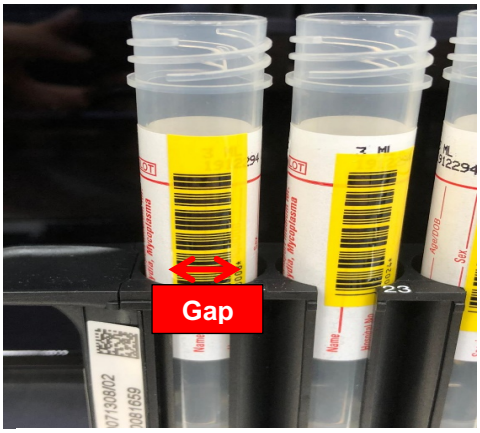
- **High quality print** (ANSI / CEN / ISO grade A or B quality) is required for the barcode label.
- Ensure that the barcode specifications & positioning meet the requirements in the table and figure below.



Label specifications

Dimension		Minimum length	Maximum length
A	Label length	N/A	80 mm
B	Code length	N/A	74 mm
C	Quiet zone	3 mm	N/A
D	Label width	12 mm	N/A
E	Code width	12 mm	N/A
F	Distance from the barcode to the label edge	N/A	1 mm

</td></tr>
</table>

Sample Tube Criteria	Description
	• Glue barcode label within a range of between 20 mm to 100 mm from the bottom of the tube and at an angle of 90° to the tube (refer to figure below).  Position of the barcode • Ensure that the orientation of the sample tube allows the barcode label to be seen through the gap as shown in the figure below. • This is to ensure that the barcode can be successfully scanned by the automated barcode scanner on the Hamilton Microlab® STAR™ instrument.  Orientation of the sample tube

5. Automated nucleic acid extraction and RT-PCR set up on the Hamilton Microlab® STAR™ instrument

The workflow on the Hamilton Microlab® STAR™ instrument is split into three stages:

- Sample transfer,
- Viral RNA Extraction, and
- RT-PCR set up.

5.1. Sample transfer

Figure 4 shows the positions of consumables / labware on the Hamilton Microlab® STAR™ platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab® STAR™ instrument software or the appendix to load all items in the correct positions.

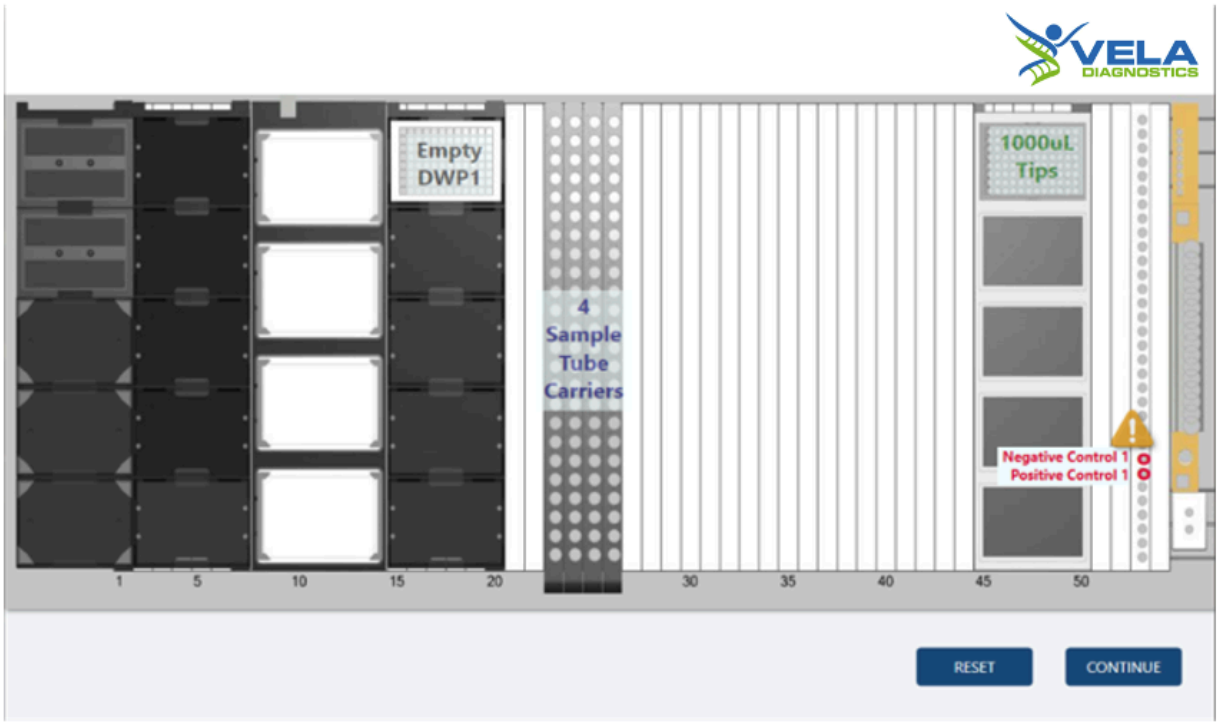


Figure 4. Layout of the Hamilton Microlab® STAR™ platform for 1 deepwell plate (1x96 tests) for sample plate preparation (refer to Appendix for 2 to 4 deepwell plates layout).

NOTE:

- Items shown are necessary for nucleic acid extraction and PCR assay set-up for application *"STAR8AL96 Vela_SampleTransfer_V1.2.med"*.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.

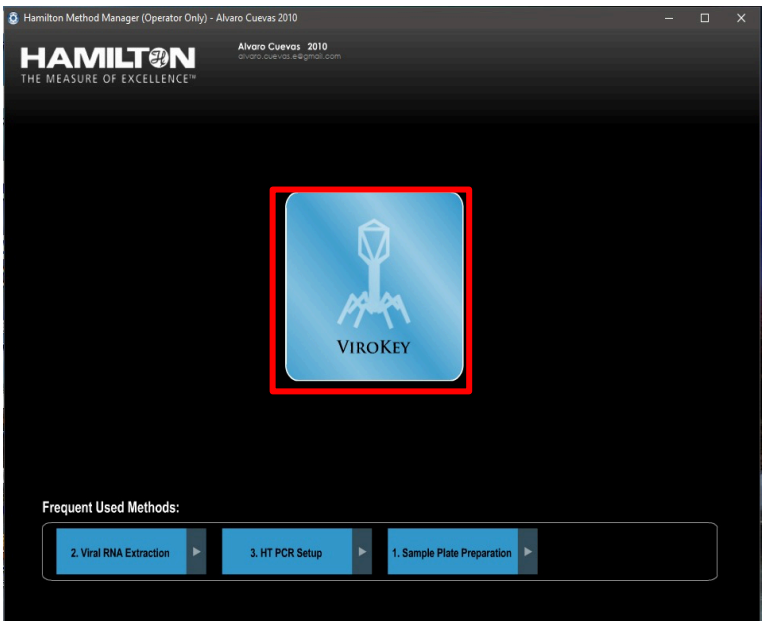
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab® STAR™ instrument user manual.

5.1.1. Switch on the computer, and wait for the initialization procedure to be completed.

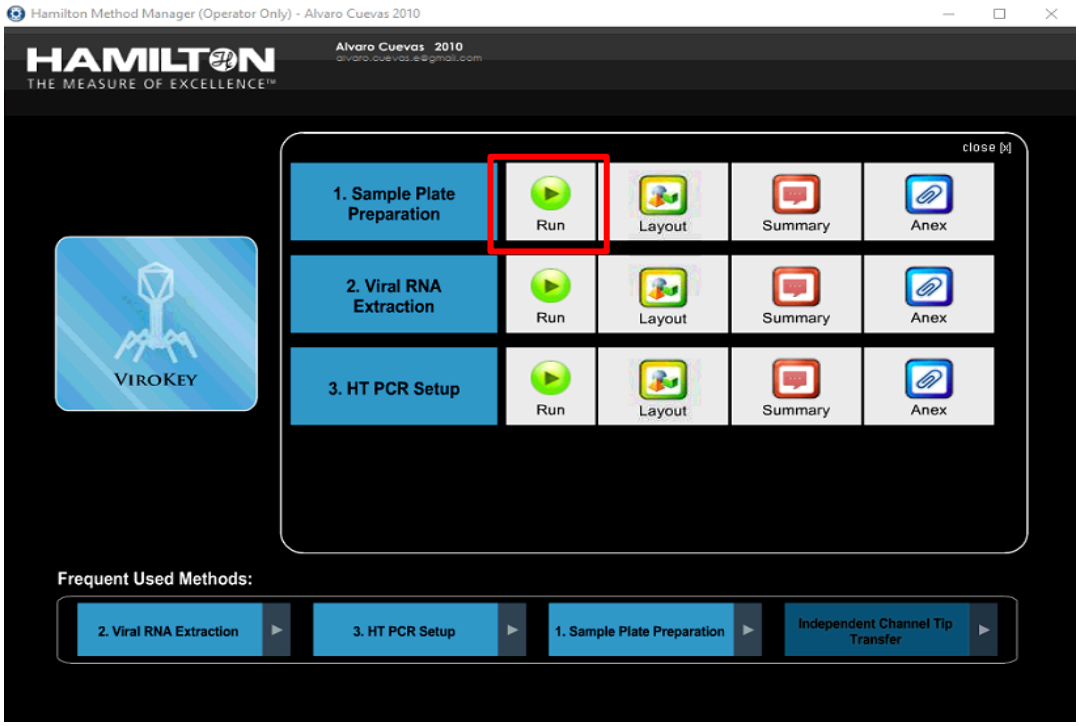
5.1.2. On the instrument's computer, launch the Hamilton Microlab® STAR™ software by double-clicking the icon.

NOTE: Please switch on Hamilton Microlab® STAR™ instrument after Hamilton Microlab® STAR™ software is launched.

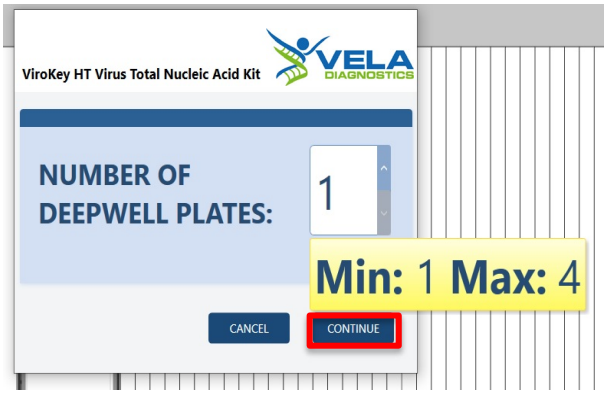
5.1.3. Press / Click "*VIROKEY*" button.



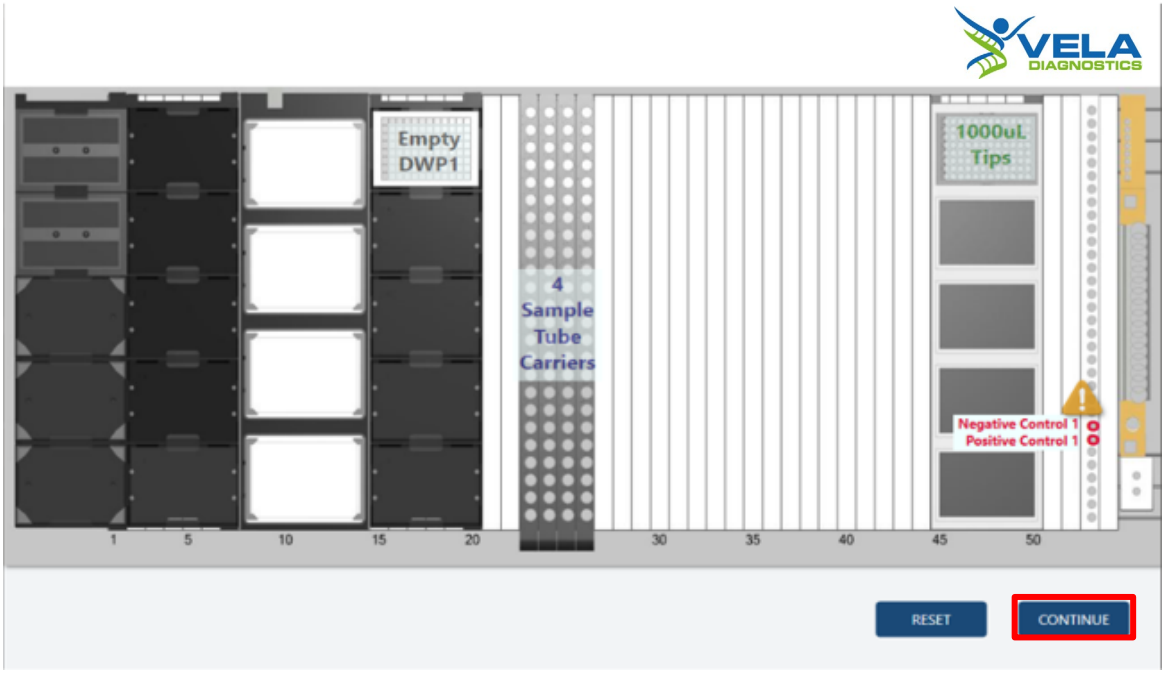
5.1.4. Press / Click the *"Run"* under *"1. Sample Plate Preparation"* to launch the sample plate preparation application.



5.1.5. Input value 1 to 4 (minimum 1 and maximum 4) for the *"NUMBER OF DEEPWELL PLATES"*. Load the empty deepwell plates onto the Hamilton Microlab® STAR™ worktable (refer to **Figure 1** in step 5.1). Press / Click *"CONTINUE"* when done.



5.1.6. Press / Click *"CONTINUE"* to proceed to scanning of NC and PC tubes.



Scan the 2D barcodes on the NC and PC for **Control Set 1 (NC1 and PC1)**. Load the **Control Set 1** onto **Tube Carrier Track 53**. Press / Click "*CONTINUE*" when done.

NOTE: Ensure that the NC and PC tubes are uncapped before loading.

5.1.7. [If applicable] Repeat step 5.1.6 for **Control Sets 2** to **4** if performing for 2x96 to 4x96 tests respectively.

NOTE: Each control set corresponds to each sample set in the table below. Each control set consists of 1 NC and 1 PC and each sample set consists of 94 samples.

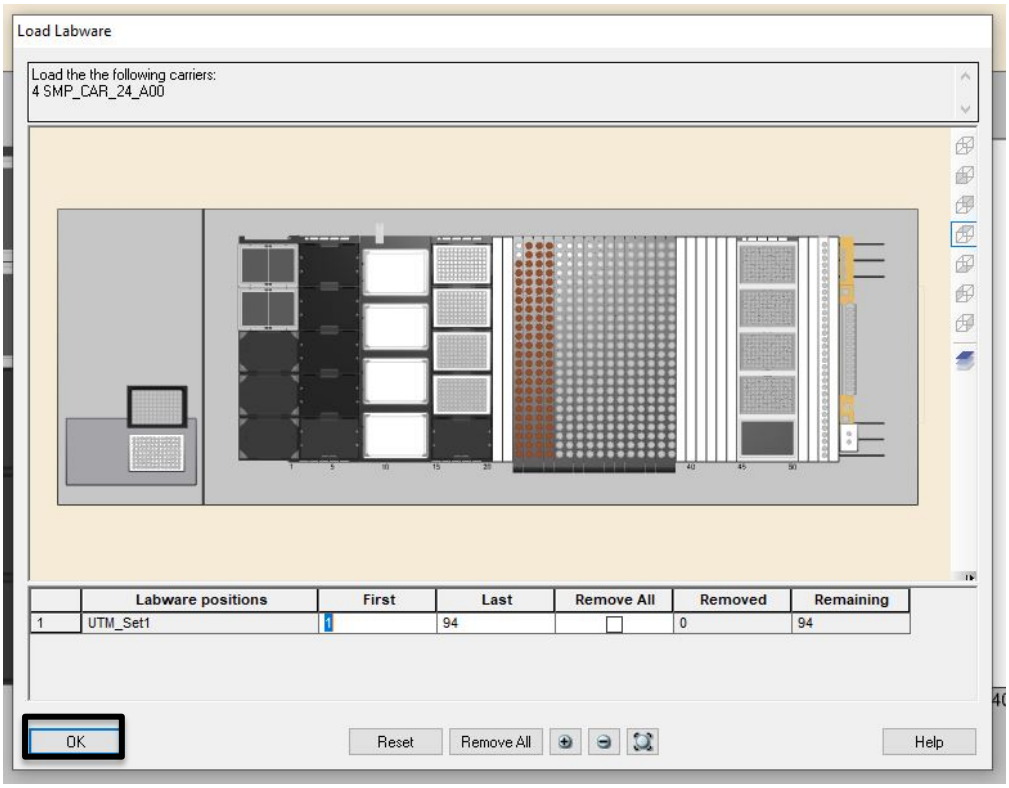
Track Carrier	Control Set	Sample Set
53	1	1
	2	2
	3	3
	4	4

5.1.8. Load the sample tubes onto the sample tube carriers.

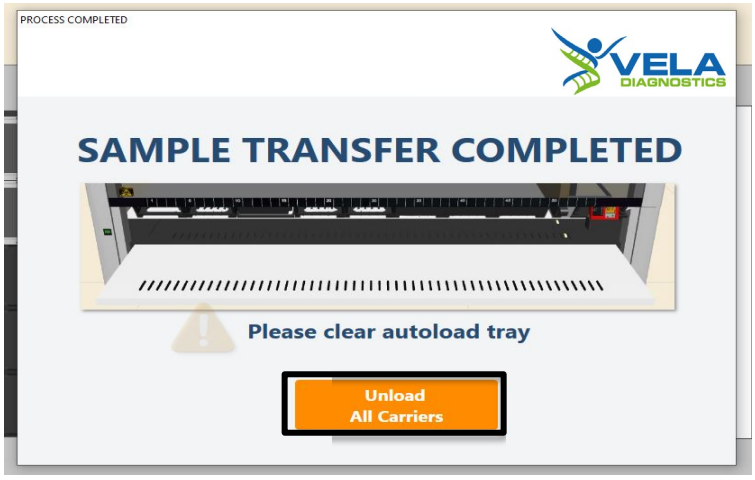
Input the number of samples used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking*" and *"dragging"* over an area). Press / Click *"OK"* when done.

NOTE:

- Ensure that the sample tubes are uncapped before loading.
- User can run less than 94 samples in each run by indicating the first and last sample in the carrier.
- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".



5.1.9. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab® STAR™ instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab® STAR™ instrument maintenance videos.



Proceed to *"2. Viral RNA Extraction",* on the *"Hamilton Method Manager"* user interface, after the run is completed.

5.2. Viral RNA Extraction

Figure 5 shows the positions of consumables / labware on the Hamilton Microlab® STAR™ platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab® STAR™ instrument software or the appendix to load all items in the correct positions.

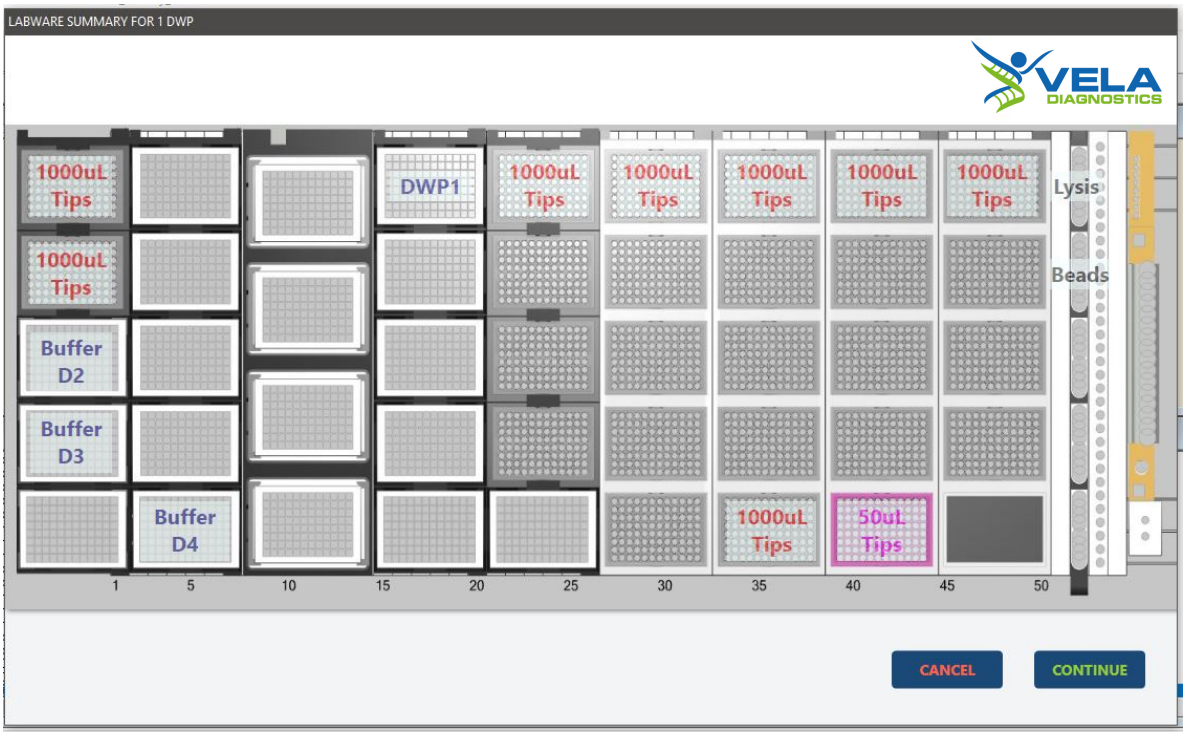
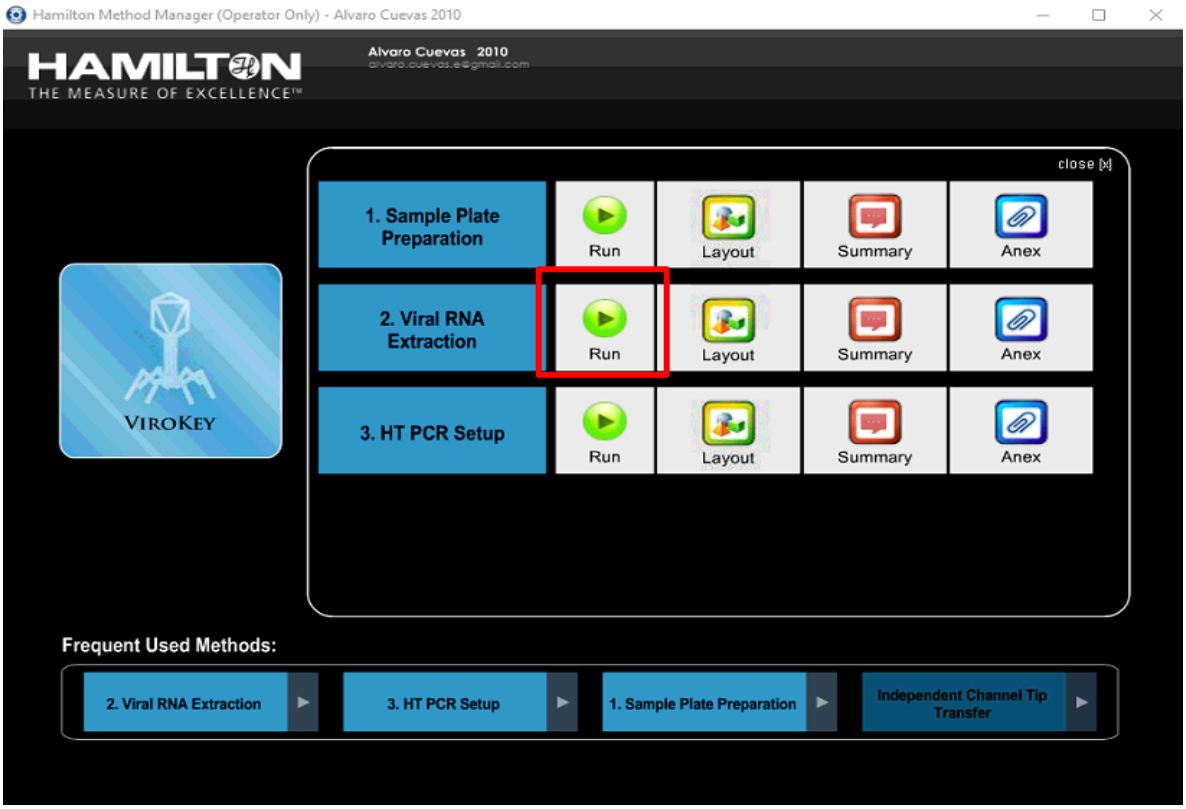


Figure 5. Layout of the Hamilton Microlab® STAR™ platform for 1 deepwell plate for viral RNA extraction (refer to Appendix for 2 to 4 deepwell plate layouts).

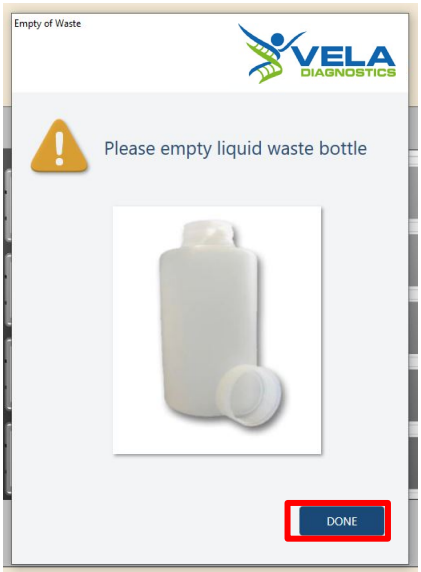
NOTE:

- Items shown are necessary for nucleic acid extraction for application *"STAR8AL96 Vela_ViroKey_V3.10.med"*.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab® STAR™ instrument user manual.

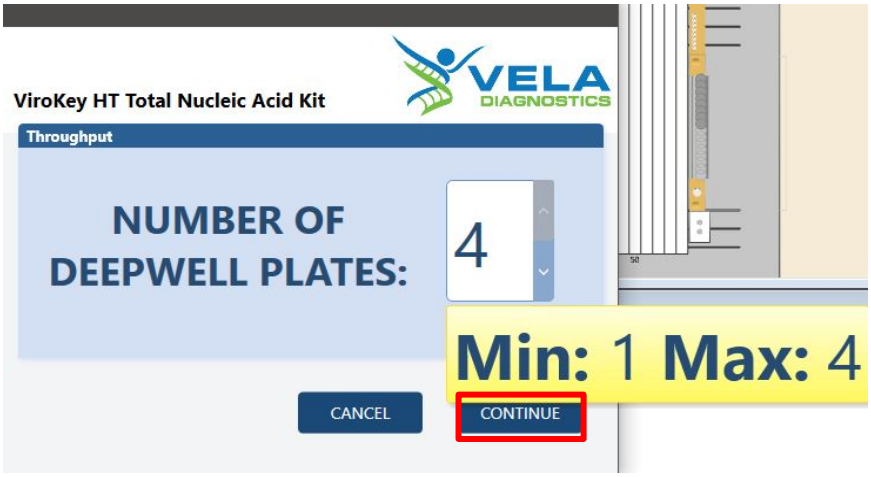
5.2.1. Press / Click the "*RUN*" under *"2. Viral RNA Extraction"*.



5.2.2. Empty the liquid waste bottle. Press / Click *"DONE"* after placing back the empty waste bottle.

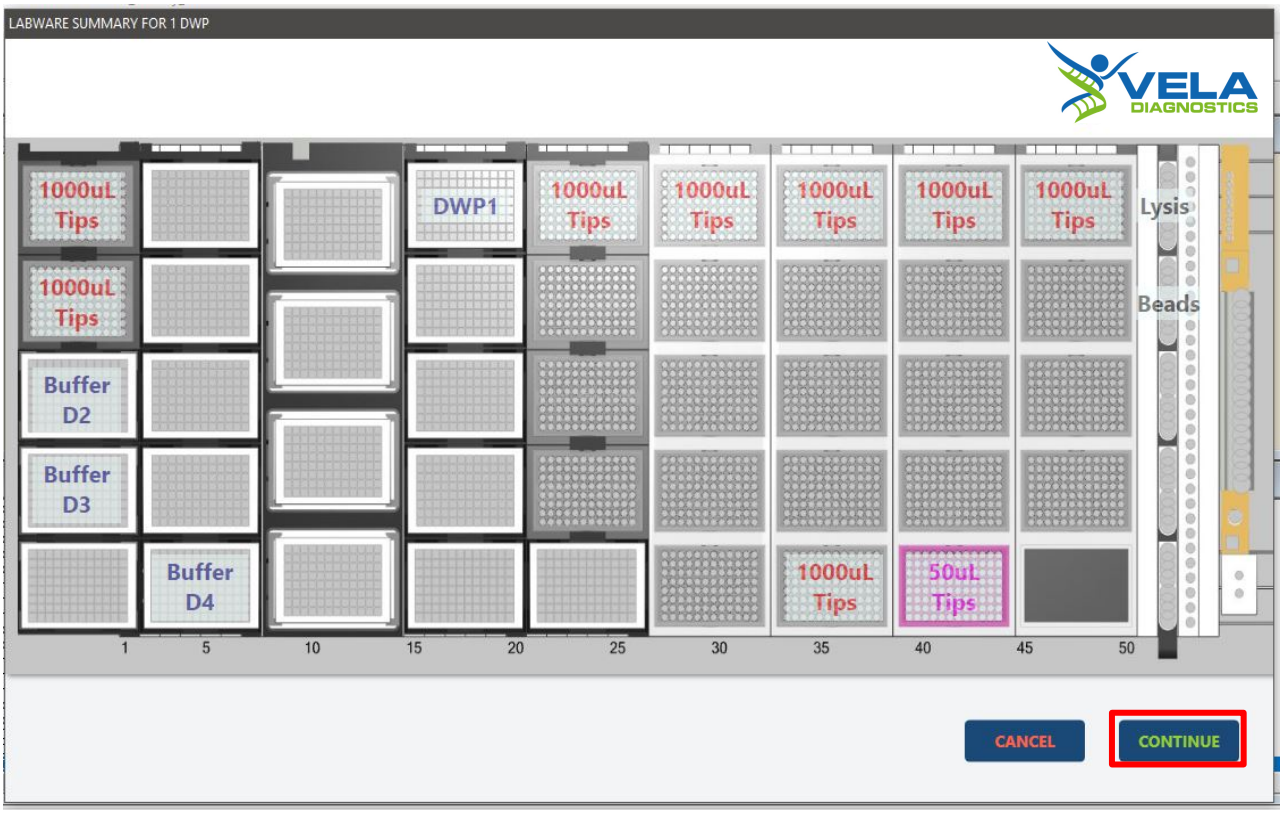


5.2.3. Input value 1 to 4 (minimum 1; maximum 4) for the *"NUMBER OF DEEPWELL PLATES"*. Press / Click *"CONTINUE"* when done.



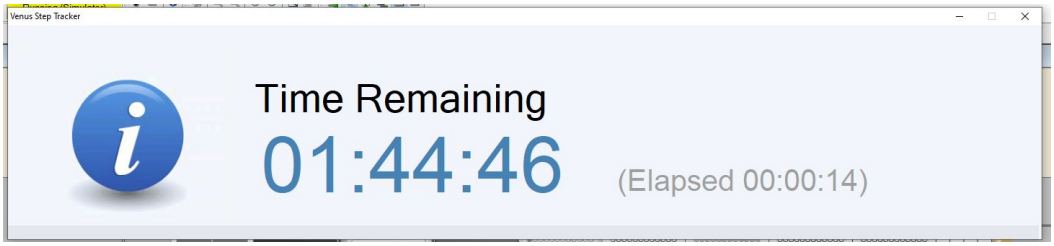
5.2.4. Prepare the reagents and consumables (Buffer D2 to D4, lysis master mix, 1,000 µL tips and 50 µL tips and beads). Press / Click *"CONTINUE"* when done. Refer to Appendix for Consumables Summary for 2 to 4 deepwell plates.

5.2.5. Load the reagents and consumables based on the layout below. Press / Click *"CONTINUE"* when done. Refer to Appendix to view layout with 2 to 4 deepwell plates.

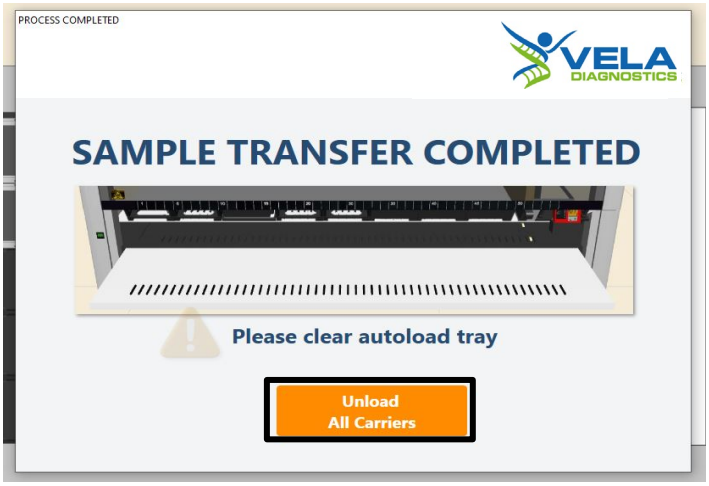


5.2.6. Scan the ViroKey® HT Total Nucleic Acid Kit label. Press / Click *"CONTINUE"* when done.

5.2.7. Wait for extraction to be completed. Extraction takes approximately 1 hour 45 minutes to complete.



5.2.8. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab® STAR™ instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab® STAR™ instrument maintenance videos.



Proceed to *"3. HT PCR Setup",* on the *"Hamilton Method Manager"* user interface, after the run is completed.

5.3. HT PCR Setup

Figure 6 shows the positions of consumables / labware on the Hamilton Microlab® STAR™ platform. Double line the *w*aste bin with biohazard bags. Please refer to the layout as indicated by the Hamilton Microlab® STAR™ instrument software or the appendix to load all items in the correct positions.

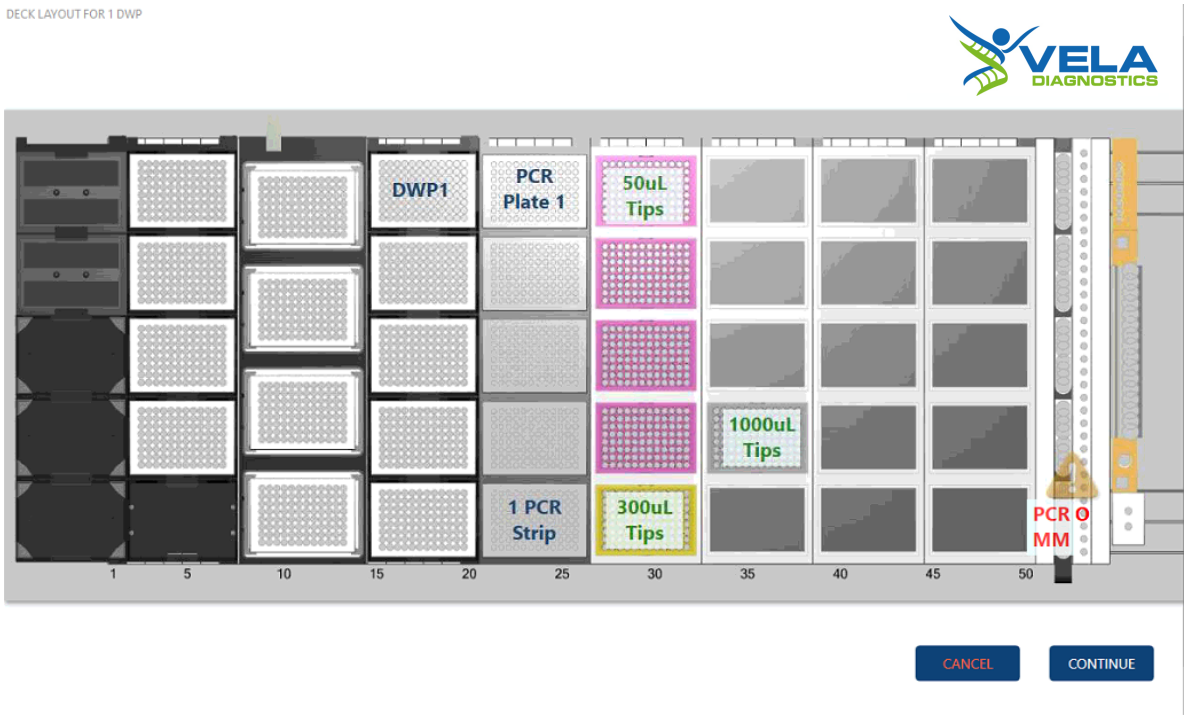
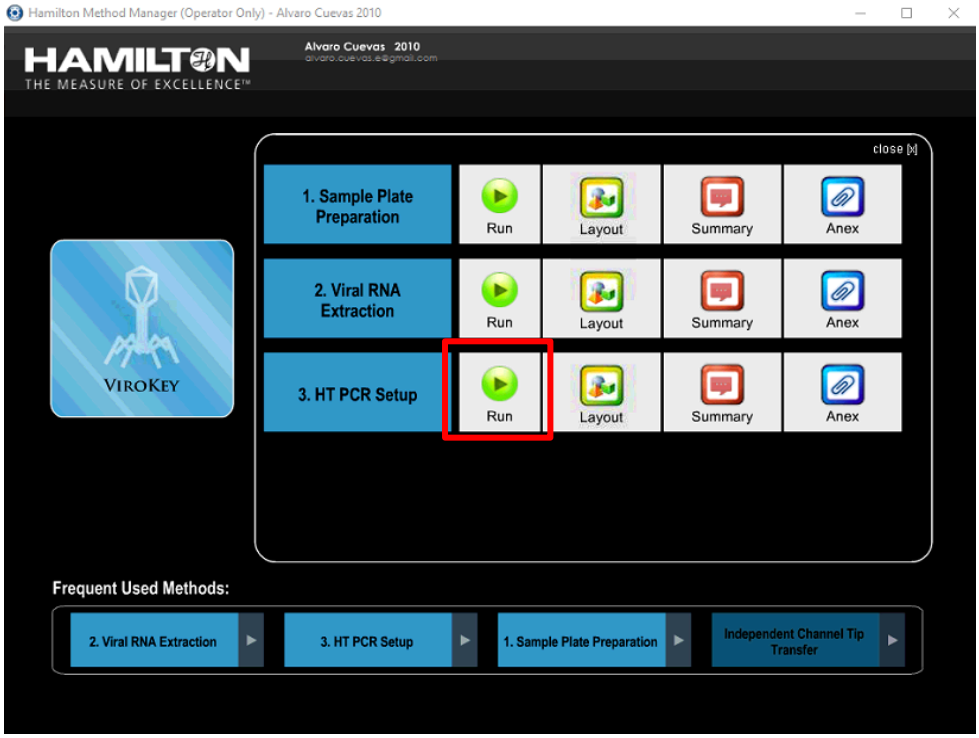


Figure 6. Layout of the Hamilton Microlab® STAR™ platform for 1 sample plate for HT PCR setup (refer to Appendix for 2 to 4 sample plate layouts).

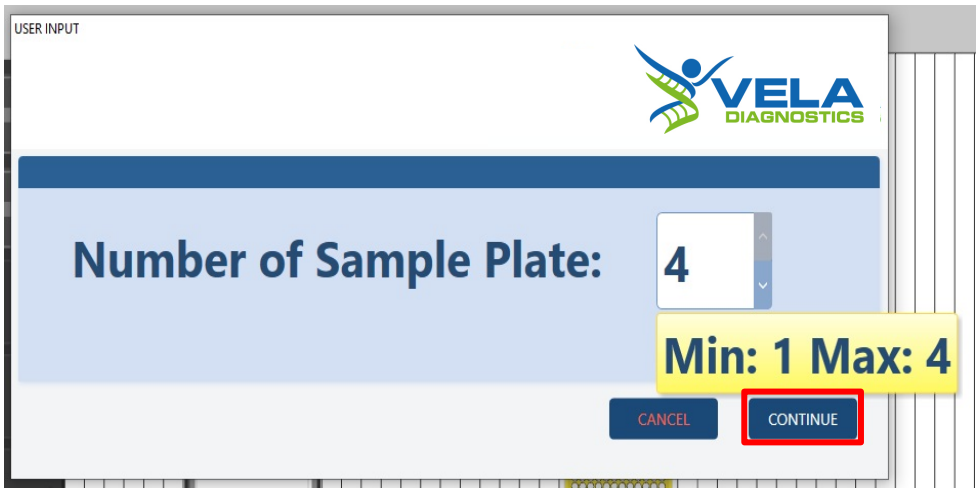
NOTE:

- Items shown are necessary for PCR assay setup for application *"STAR8AL96 Vela_PCRsetup_V1.3.med"*.
- Ensure all consumables / labware are properly placed, aligned and secured into their respective positions.
- Ensure that the biohazard bags are properly attached to the waste bin before starting a protocol run. For more information, please refer to the Hamilton Microlab® STAR™ instrument user manual.

5.3.1. Press / Click the "*RUN*" under *"3. HT PCR Setup"*.

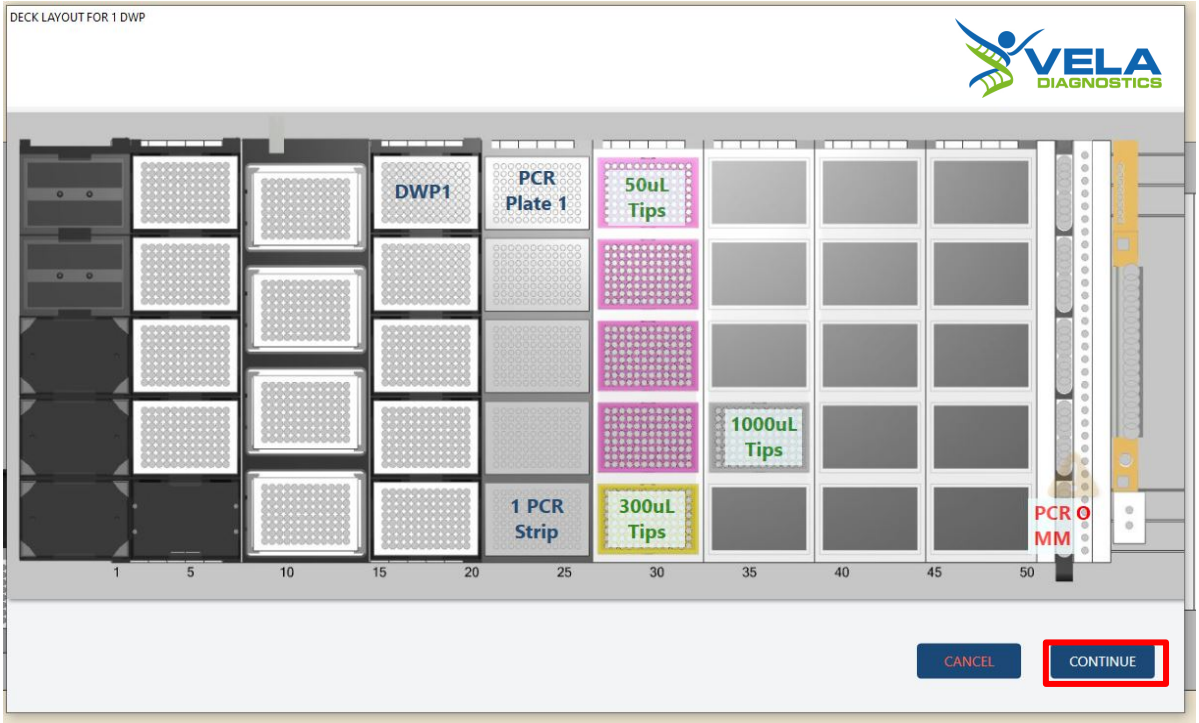


5.3.2. Input value 1 to 4 (minimum 1; maximum 4) for the *"Number of Sample Plate"*.



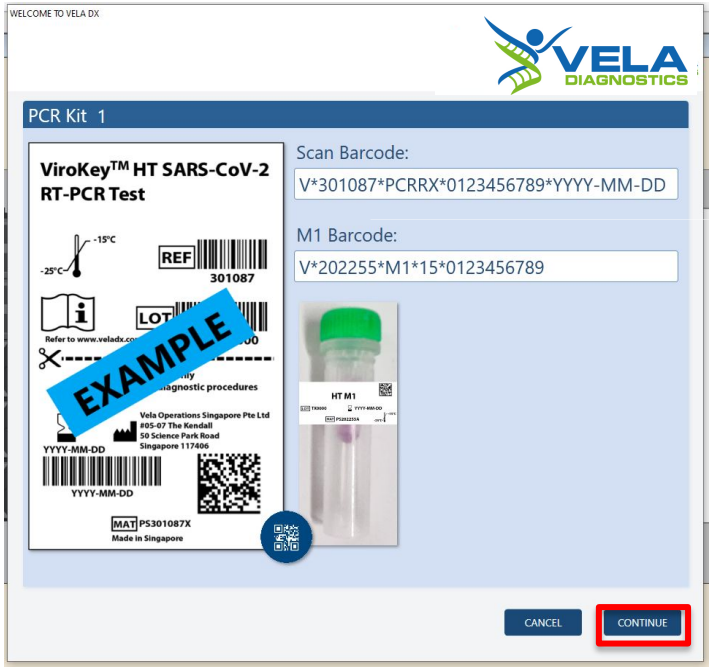
Press / Click *"CONTINUE"* when done.

5.3.3. Load the reagents and consumables based on the layout below. Press / Click *"CONTINUE"* when done. Refer to Appendix to view layout with 2 to 4 sample plates.



NOTE: PCR Strip in the layout refers to HT 8-Strip Tubes.

5.3.4. Scan the PCR Kit 1 (HT M1 of ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) kit) label. Press / Click *"CONTINUE"* when done.



5.3.5. Scan the PCR Plate 1 label. Place plate on PCR plate carrier, Track 21 – 26, Position 1. Press / Click *"CONTINUE"* when done.

5.3.6. [If applicable] Repeat step 5.3.4 to step 5.3.5 and for **PCR Plates 2** to **4** if performing for 2x96 to 4x96 tests respectively.

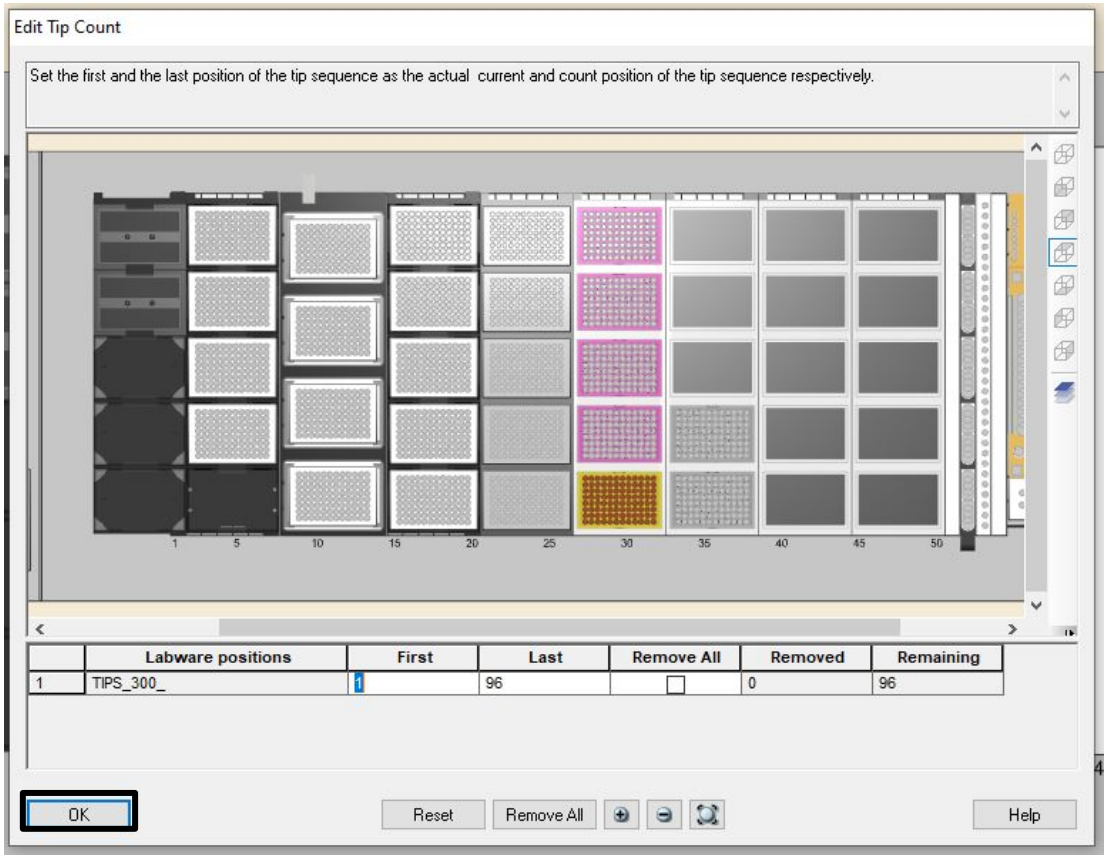
NOTE:

Track	PCR Plate	Position
21 – 26	1	1
	2	2
	3	3
	4	4

5.3.7. Input the number of 300 µL tips used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking*", *"holding"* and *"dragging"* over an area). Press / Click *"OK"* when done.

NOTE:

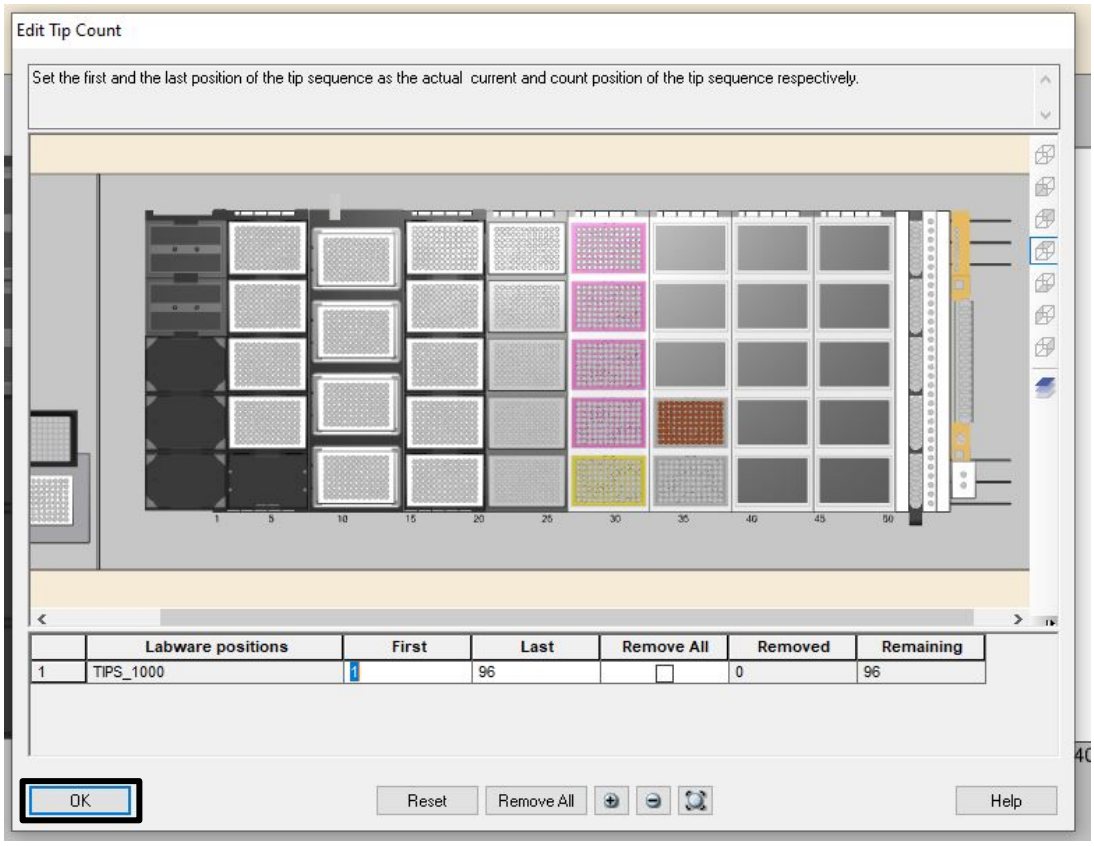
- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".
- Remove the leftover tips (if any) and use them for the next PCR setup.



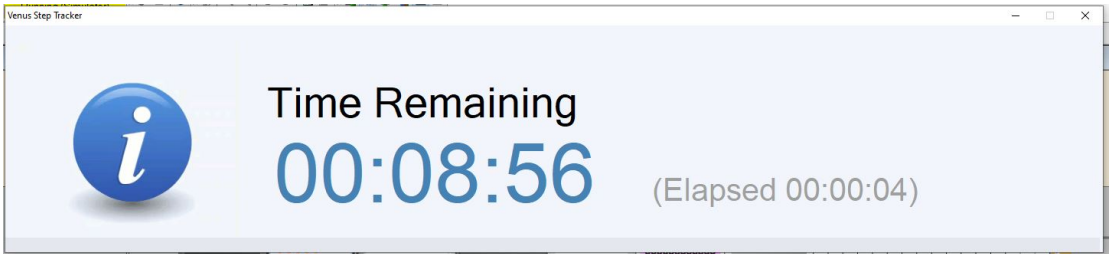
5.3.8. Input the number of 1,000 µL tips used under the *"First"* and *"Last"* columns or select the area where the *"First"* and *"Last"* samples are placed using the cursor (by *"clicking*" and *"dragging"* over an area). Press / Click *"OK"* when done.

NOTE:

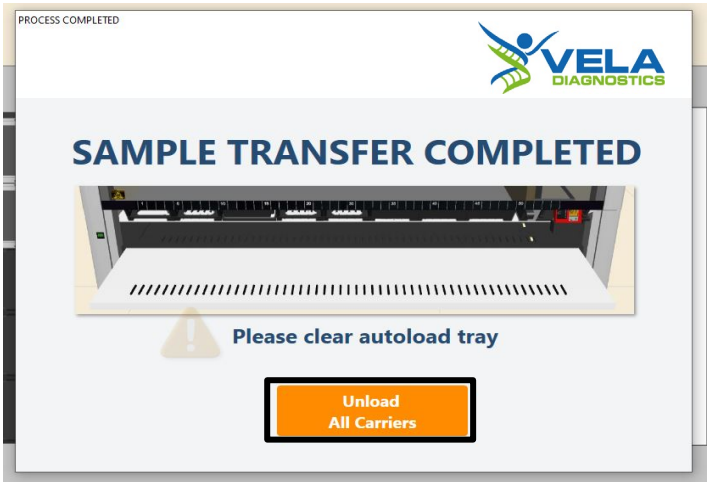
- After the user has indicated the *"First"* and *"Last"* samples, location of the samples are shown as "brown circles".
- Remove the leftover tips (if any) and use them for the next PCR setup.



5.3.9. Wait for PCR setup to be completed. PCR setup takes approximately 9 minutes to complete.



5.3.10. Press / Click *"Unload All Carriers"* after the run is completed. Unload all carriers, clean and disinfect the Hamilton Microlab® STAR™ instrument after each run. For instrument maintenance, please refer to the Hamilton Microlab® STAR™ instrument maintenance videos.



After the run is completed, carefully remove the MicroAmp® Fast Optical 96-Well Reaction Plate. Apply the MicroAmp® Optical Adhesive Film over the plate and seal the plate tightly to prevent contamination. Briefly spin down the PCR plate and load it onto the *Sentosa*® SA201 Real-Time PCR Instrument. Proceed to RT-PCR and data analysis using the *Sentosa*® SA201 Real-Time PCR Instrument and *Sentosa*® SA201 Reporter (page 91 to page 105).

After RT-PCR and data analysis are complete, proceed to page 12 for *"Result interpretation"*.

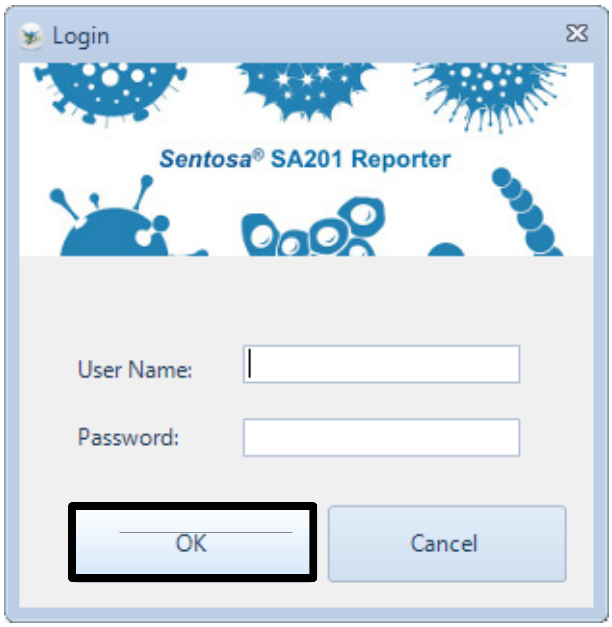
PCR and data analysis using the *Sentosa*® SA201 Real-Time PCR Instrument and *Sentosa*® SA201 Reporter software

6. PCR on the Sentosa® SA201 Real-Time PCR Instrument

6.1. Switch on the *Sentosa*® SA201 Real-Time PCR Instrument by pressing the power button on the instrument.
NOTE: Ensure the green indicator is lit and not flashing.

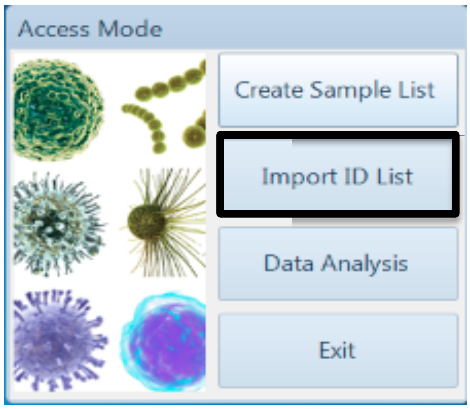
6.2. Launch the *Sentosa*® SA201 Reporter software by double-clicking on the icon.

Type the user name and password, and then click *"OK"*.



6.3. After logging in, "*Access Mode*" pop-up dialog is displayed.

Click *"Import ID List"* under *"Access Mode"* pop-up dialog to import the required *".smp"* file generated by the *Sentosa*® SX101 instrument (**Figure 7**) / Hamilton Microlab® STAR™ instrument (**Figure 8**).



6.3.1. Import *".smp"* file generated by the *Sentosa*® SX101 instrument

Select *".smp"* file to import from *"Sentosa SA > SMP"* folder. Click *"Open"* to import the selected file.

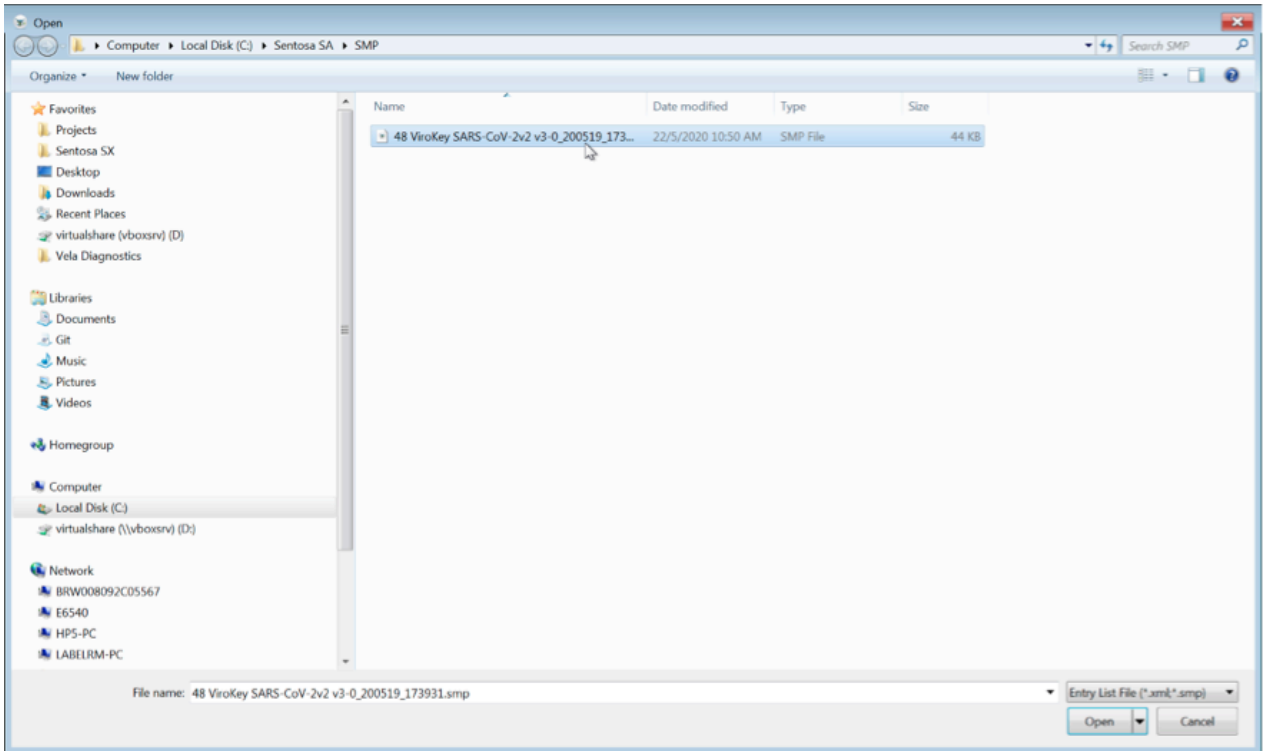


Figure 7. *".smp"* file generated by *Sentosa*® SX101 instrument is found in *"Sentosa SA > SMP"* folder.

6.3.2. Import *".smp"* file generated by the Hamilton Microlab® STAR™ instrument

Select *".smp"* file to import from *"Veladx"* folder. Click *"Open"* to import the selected file.

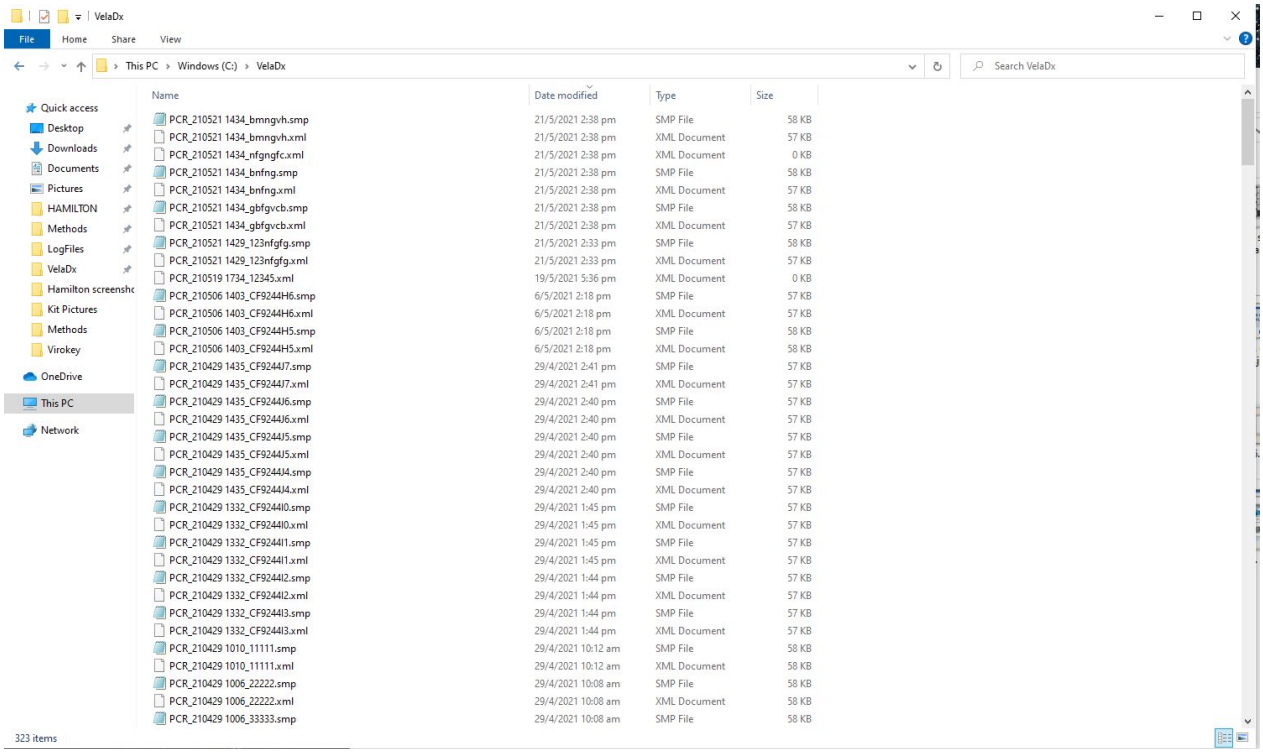
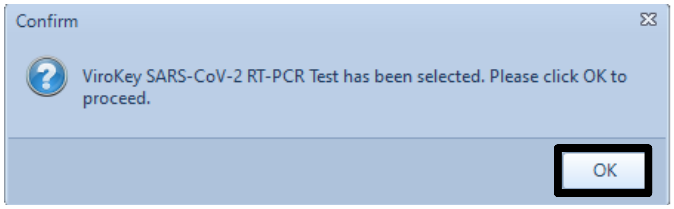
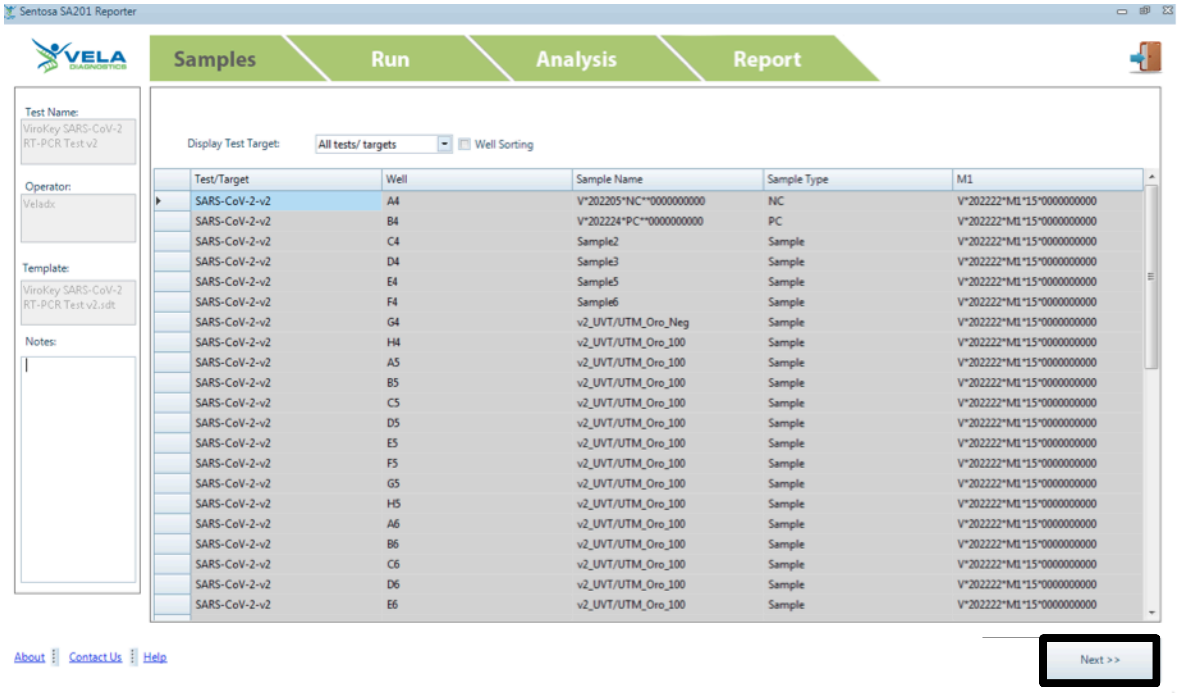


Figure 8. *".smp"* file generated by Hamilton Microlab® STAR™ instrument is found in *"Veladx"* folder.

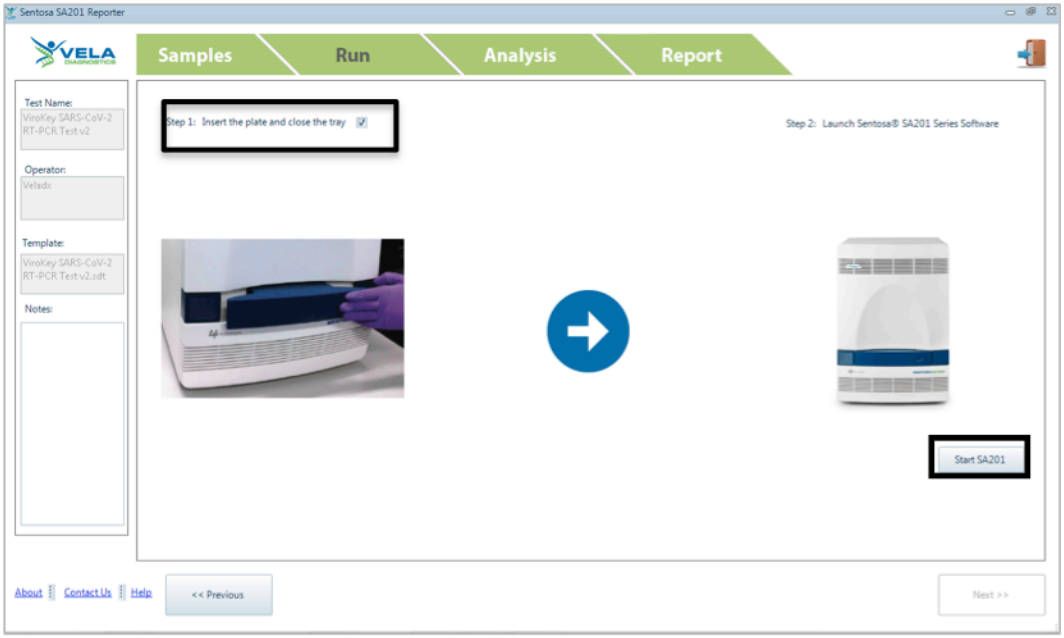
6.4. After the *".smp"* file has been imported, the software will require a confirmation on the selected assay. Click "*OK*" to confirm the sample layout and the information displayed from the "*.smp*" file.



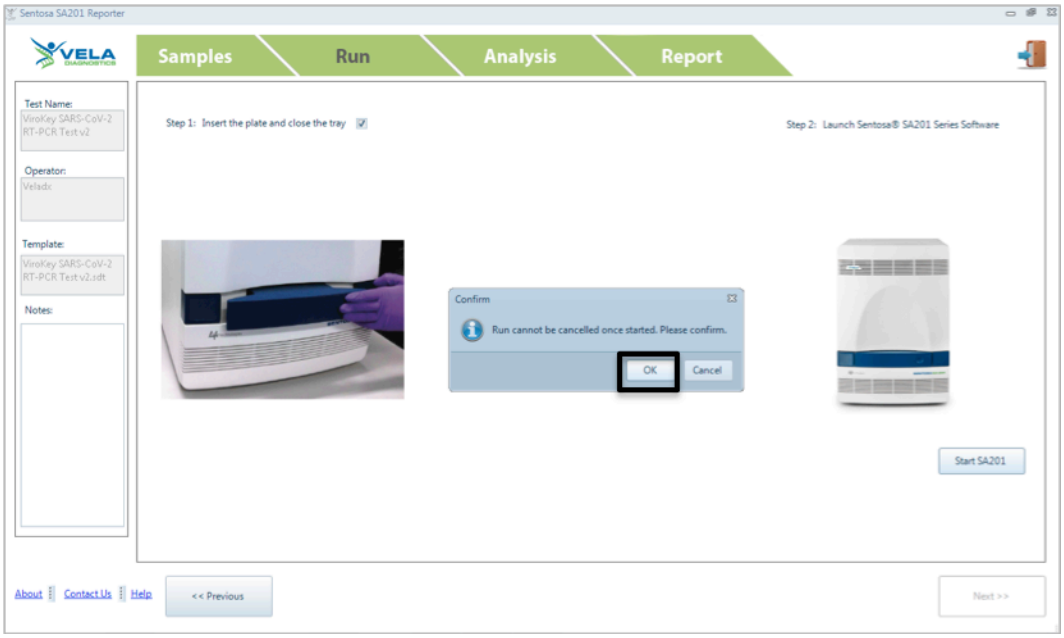


Click *"Next"*.

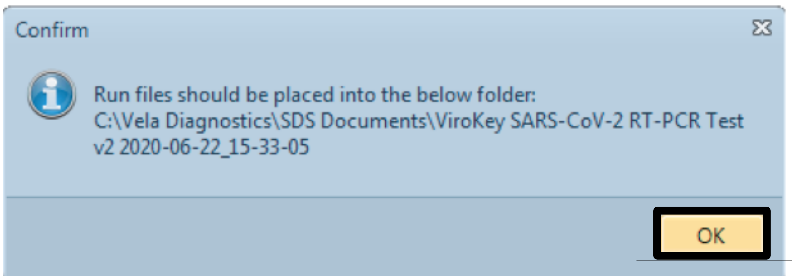
6.5. Activate "*Insert the plate and close the tray*" checkbox to activate *"Step 2: Launch Sentosa® SA201 Series Software"*. Click *"Start SA201"* button.



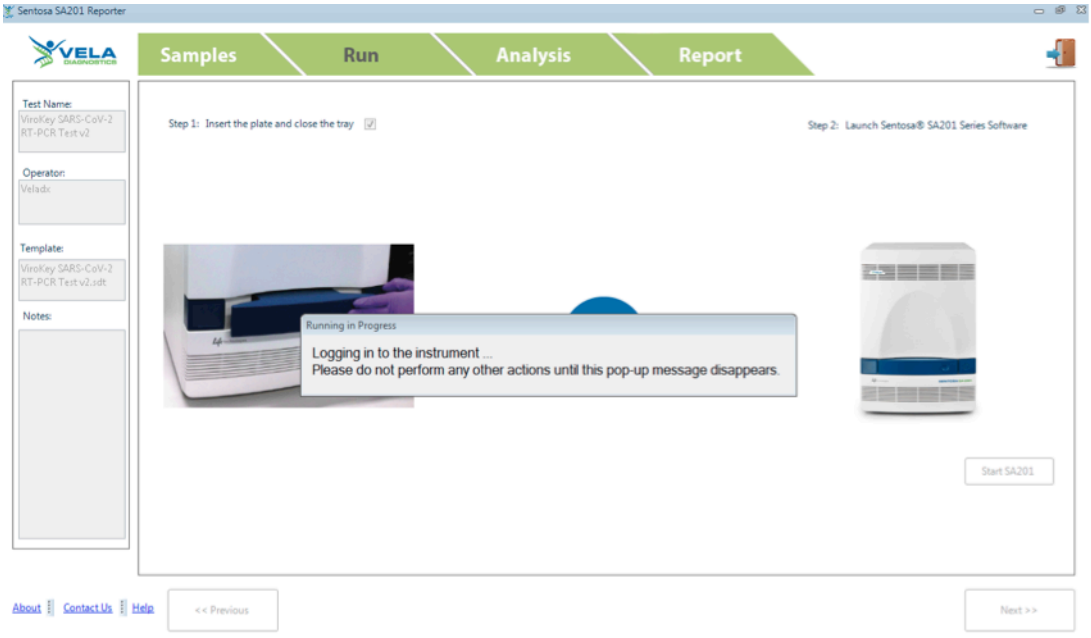
6.6. A "*Confirm*" dialog box will be displayed. Click "*OK*".



6.7. Another *"Confirm"* dialog box will be displayed. Click *"OK"*.



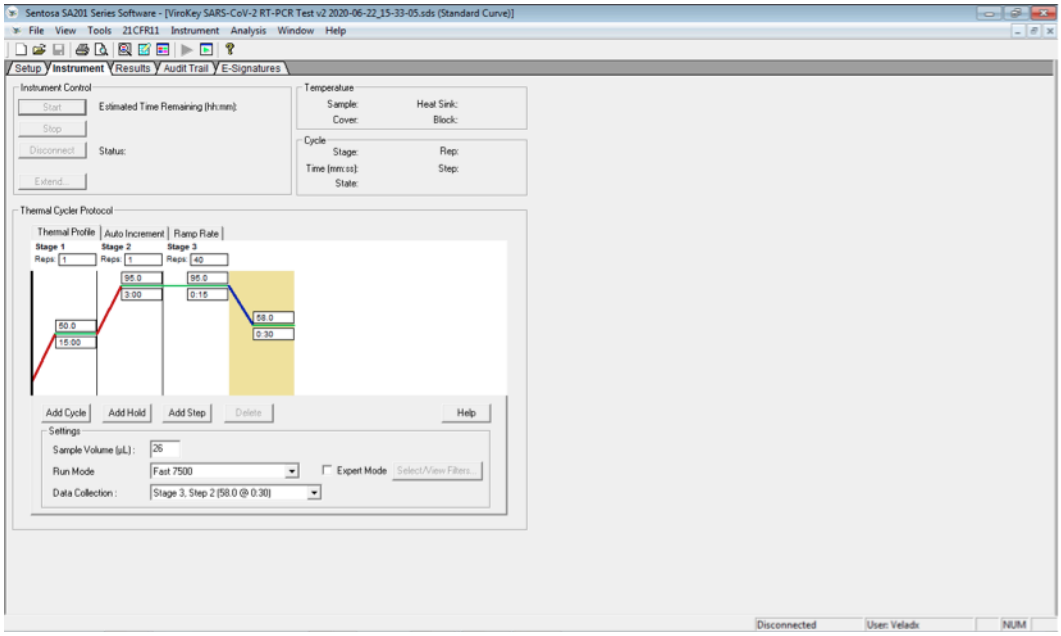
6.8. The *Sentosa*® SA201 Reporter will begin the process (automatically) of logging in to the *Sentosa*® SA201 Series Software and running a series of steps on it to start the new run. A pop-up window titled *"Running in Progress"* will be displayed throughout this process, reminding the user not to perform any other actions. The pop-up window will also indicate the individual specific steps that are being performed in the background.



The following is the list of automated steps that will be performed on the *Sentosa*® SA201 Series Software:

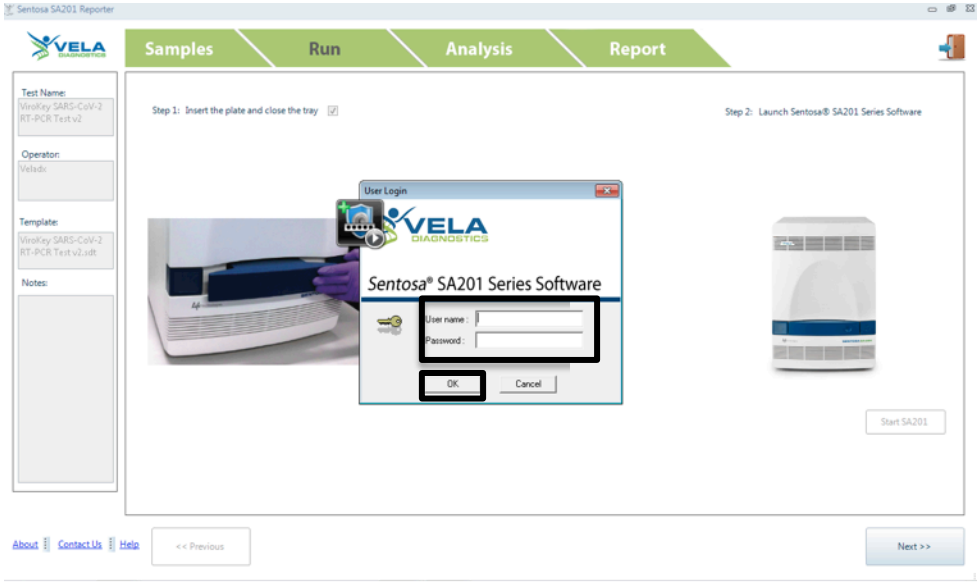
I. Logging in to the *Sentosa*® SA201 Series Software using the same user account credentials that were used to log in to the *Sentosa*® SA201 Reporter.
II. Importing the sample setup file which was generated by the *Sentosa*® SA201 Reporter.
III. Saving the run document (*".sds"*) file.
IV. Starting the run.

Please do not perform any other action during the automated process. The run will be started on the *Sentosa*® SA201 instrument at the end of the process. This can be verified by selecting the *"Instrument"* tab and checking the *"Instrument Control"* panel on the *Sentosa*® SA201 Series Software.

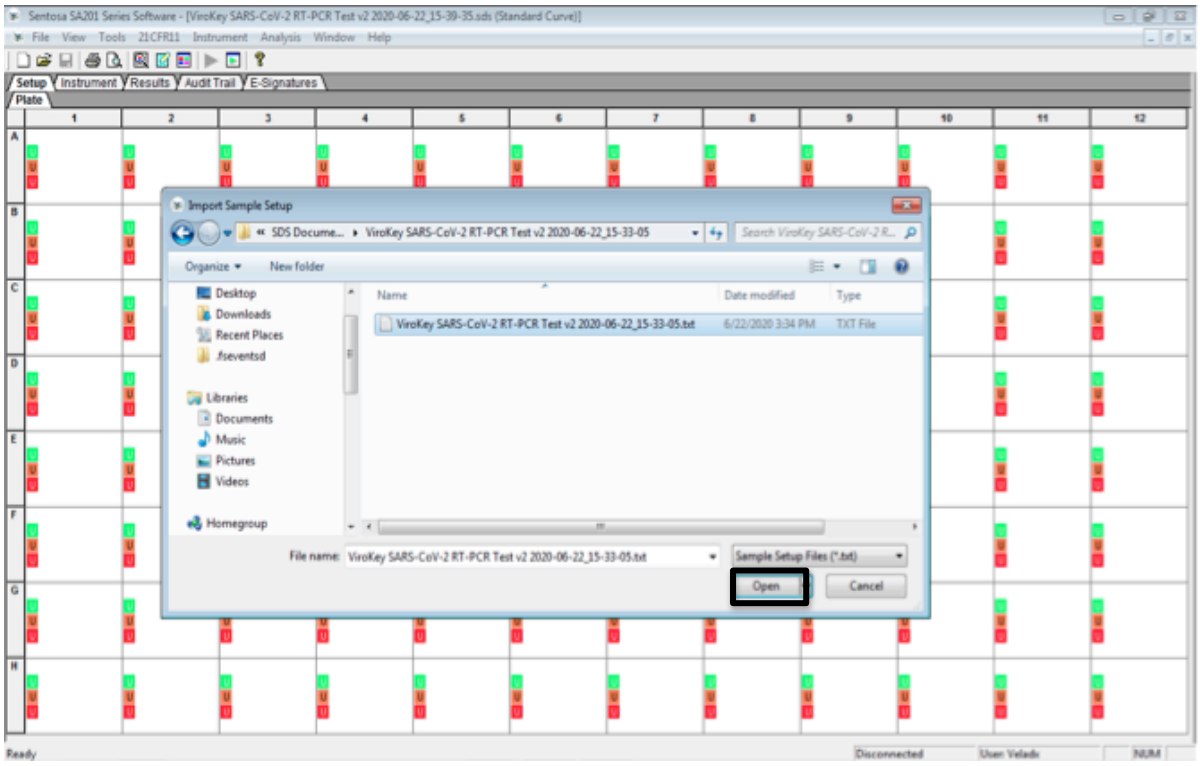


6.9. [Optional] If the automated process is stopped or interrupted due to any unforeseen circumstances, the run can be resumed manually by following the steps listed below. Please skip any steps that have already been completed by the automated process before the interruption occurs.

I. Log in to the *Sentosa*® SA201 Series Software by typing the user name and password in the *"User Login"* pop-up window. Click *"OK"*.



II. Select *"File"* from the main menu and *"Import Sample Setup"* from the drop-down list. *"Import Sample Setup"* pop-up window will be displayed. The sample file is given a default file name *("Sentosa SA201 Assay name PCR/RT-PCR Test YYYY-MM-DD_HH-MM-SS.txt"*). Select the *".txt"* file and click *"Open"*.
NOTE: DO NOT modify the generated *".txt"* file as this will result in an error.



The sample layout will change according to the imported sample setup file.

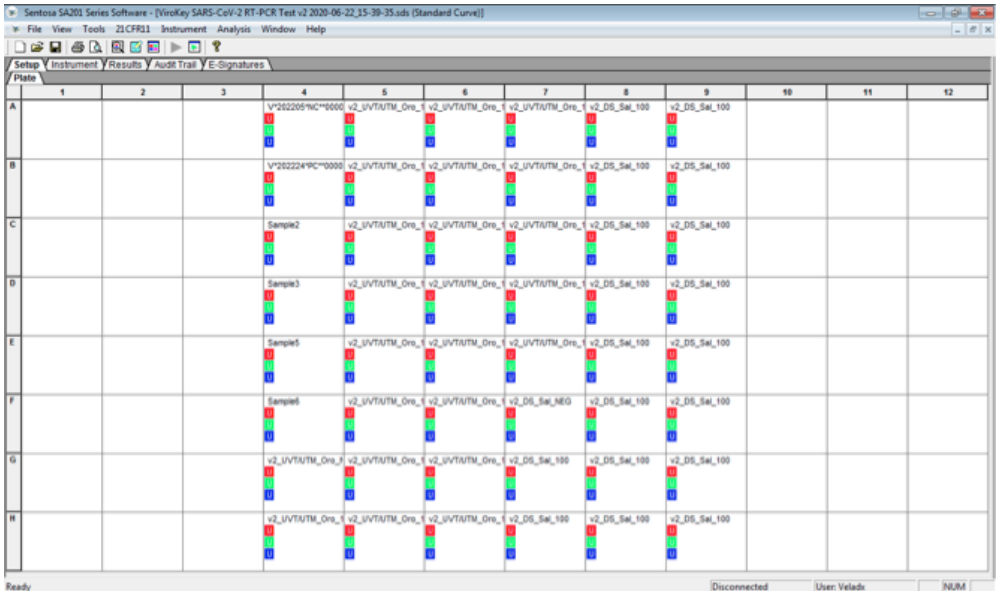


Figure 9. Imported sample setup file for ViroKey® SARS-CoV-2 RT-PCR Test (8x48).

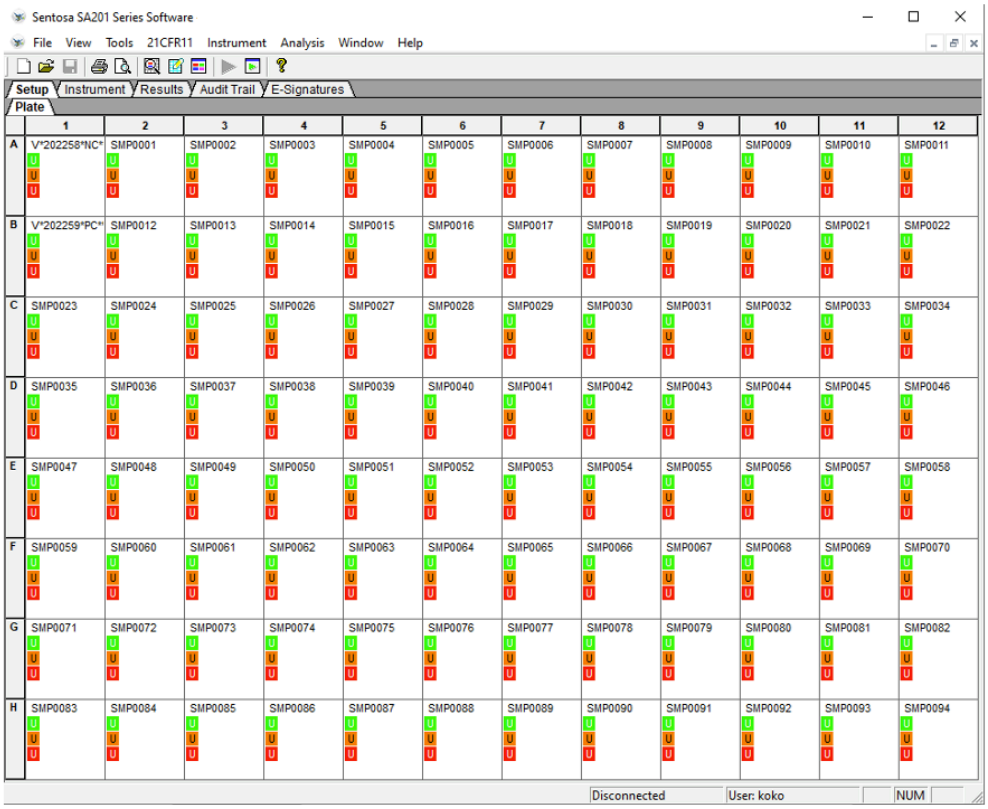
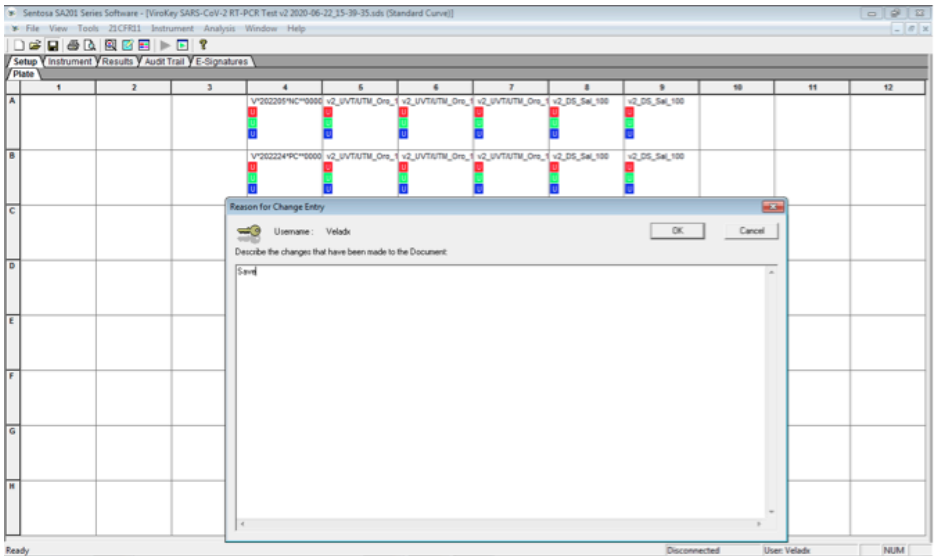
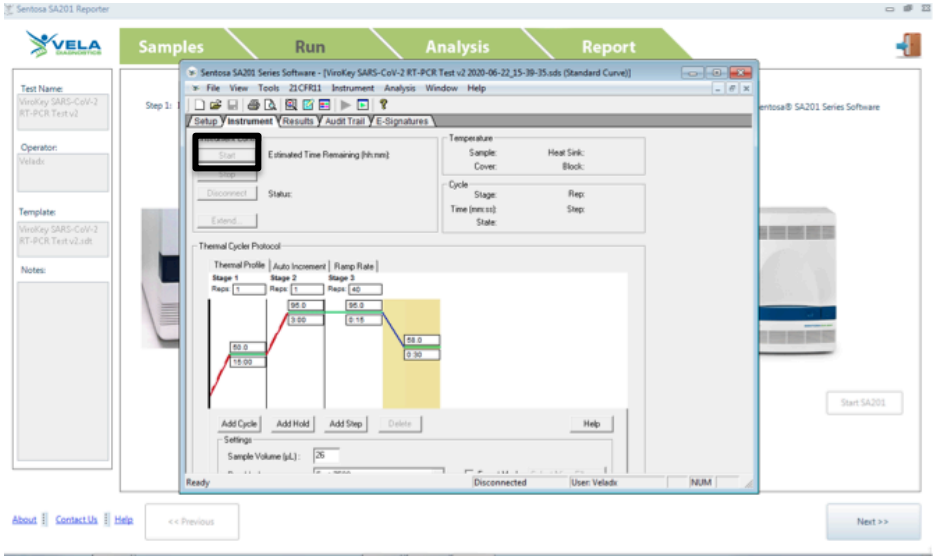


Figure 10. Imported sample setup file for ViroKey® SARS-CoV-2 RT-PCR Test (4x96).

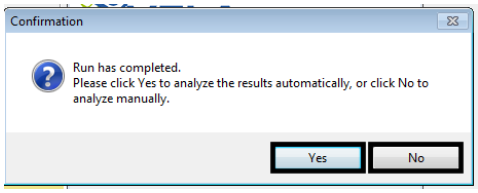
III. Select *"File"* from the main menu and *"Save"* from the drop-down list. *"Reason for Change Entry"* window will be displayed. Describe the changes made in the document or simply enter *"Save file"* or any other suitable text in the textbox and click *"OK"* to save the run document (*".sds"*) file.
NOTE: DO NOT modify the generated *".sds"* file as this will result in an error.



IV. Select the *"Instrument"* tab and click *"Start"* to start the run.



6.10. At the end of the run, a confirmation pop-up window will be displayed indicating that the run has completed. A window will prompt the user if result analysis on the *Sentosa*® SA201 Series Software is to be performed automatically or manually.

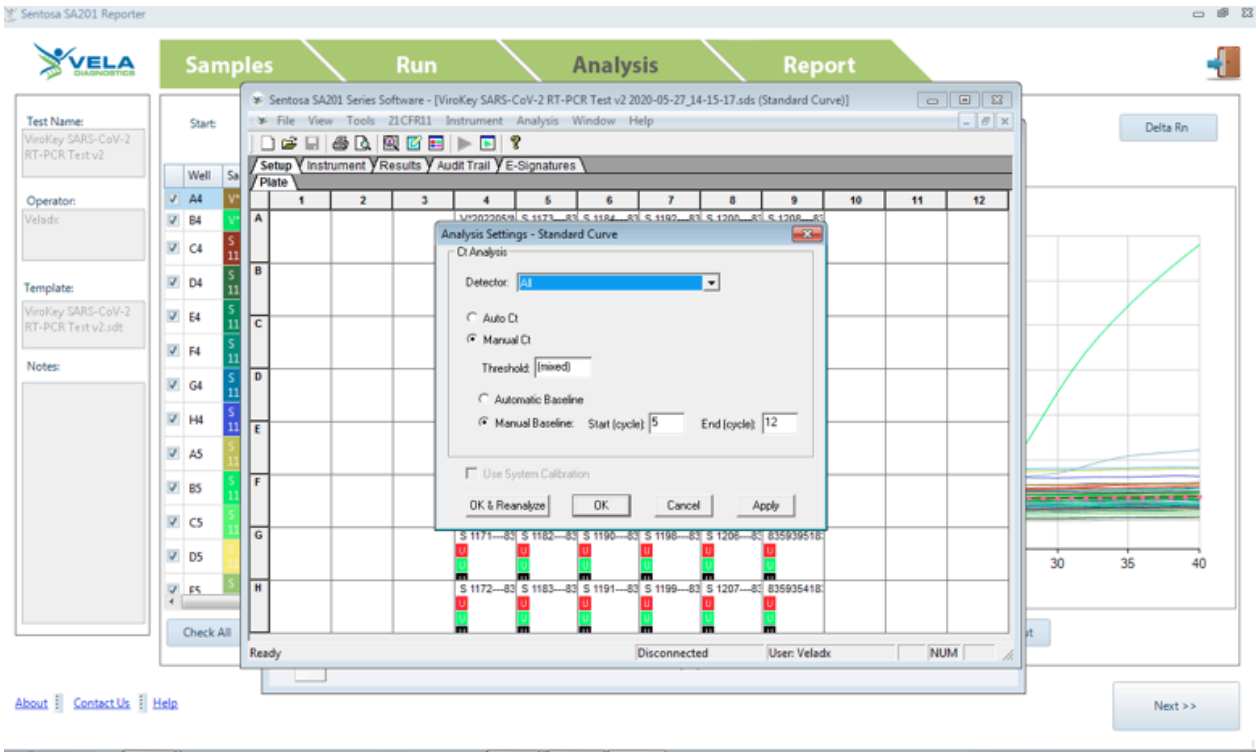


Click *"Yes"* to perform the result analysis automatically by using preconfigured analysis settings. A pop-up window titled *"Analysis in Progress"* will be displayed briefly, reminding the user not to perform any other actions.

The pop-up window will disappear when the result analysis has completed successfully. The *Sentosa*® SA201 Series Software may now be closed by clicking the close button *"*  *"* on the title bar. On the *Sentosa*® SA201 Reporter, click *"Next"* to proceed to data analysis.

[Optional] In the previous step, click *"No"* if the user wishes to make changes to preconfigured analysis setting(s) before performing the result analysis. Click *"OK"* on the pop-up window with the message *"The run completed successfully"*. Log in to the *Sentosa*® SA201 Series Software if *"Idle Timeout re-authentication required"* pop-up window is prompted.

On the *Sentosa*® SA201 Series Software, change the analysis setting(s) as necessary. Select *"Analysis"* from the main menu and *"Analyze"* from the drop-down list. *"Reason for Change Entry"* window will be displayed. Enter *"Analysis"* or any other suitable text in the text box and click *"OK"* to perform the result analysis.



The *Sentosa*® SA201 Series Software may now be closed by clicking the close button "" on the title bar. On the *Sentosa*® SA201 Reporter, click *"Next"* to proceed to data analysis.

7. *Automated data analysis on Sentosa® SA201 Reporter*

Ct values for each channel will be displayed under *"Analysis"* tab. Click *"Next"* to switch to *"Report"* tab to generate report.

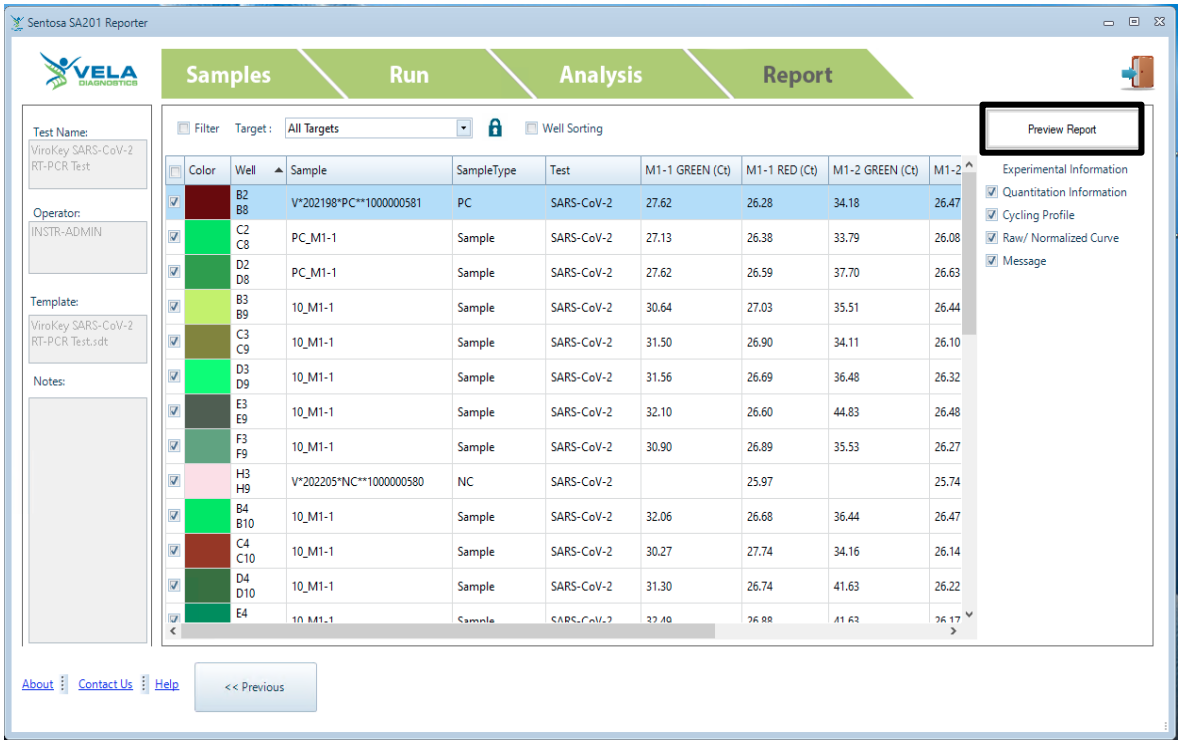
Under *"Analysis"* tab, there are other functions available as described below:

- Delta Rn is the magnitude of the fluorescence signal generated during the PCR at each time point. Delta Rn curve represents the signal processed from the raw data.
- Click *"Linear Scale"* to display the Y-axis in linear scale.
- Click *"Log Scale"* to display the Y-axis in log scale.
- Click *"Check All"* / *"Uncheck All"* to select / unselect all samples from the list. Only the selected sample curves will be displayed.
- To enlarge a particular area of a sample curve, click *"Zoom In"* and select the area of interest. Click *"Zoom Out"* to decrease the magnification of the curve.
- Click *"Auto-Scale"* to display all selected sample curves automatically to a standard size.

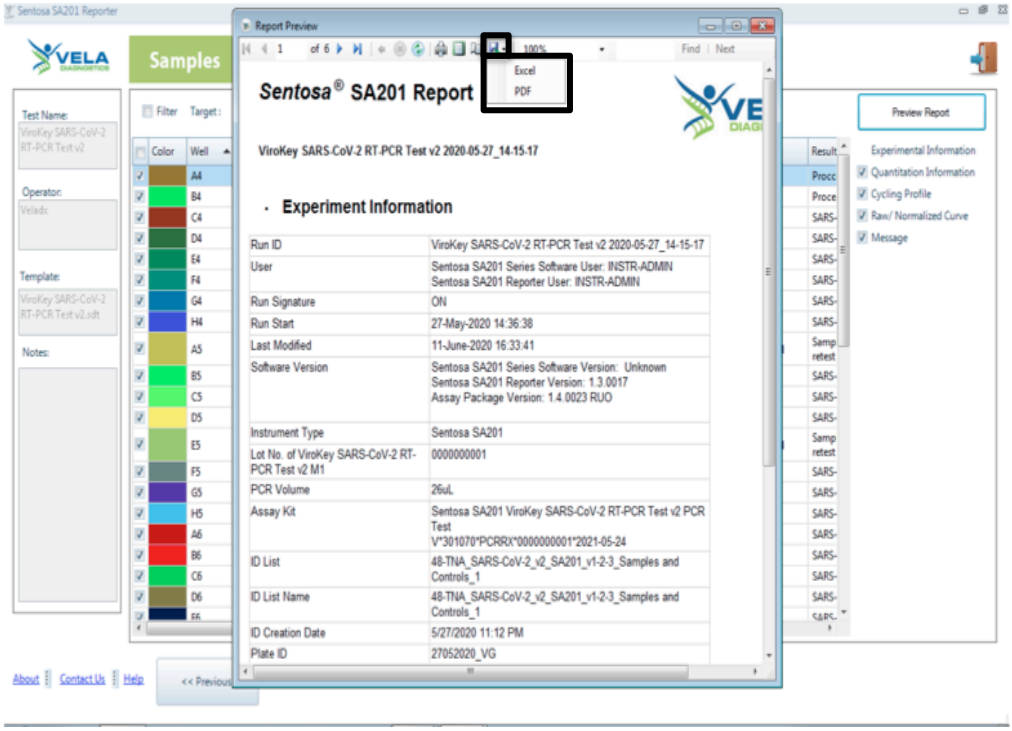
7.1. Report generation

Under *"Report"* tab, there are other functions available as mentioned below:

- Activate *"Filter"* checkbox to display the selected samples, or deactivate *"Filter"* checkbox to hide the selected samples. User can also select individual samples by activating the checkbox for each sample. Only selected samples will be displayed in the report.
- Click "  " icon to group the samples with same sample ID. If user selects one sample ID, all the grouped samples will be selected together.
- Activate the checkboxes under *"Experimental Information"* to allow the user to customize the report.
  - Activate *"Quantitation Information"* checkbox to display assay analysis settings for each fluorescence channel.
  - Activate *"Cycling Profile"* checkbox to display the run parameter settings.
  - Activate *"Raw / Normalized Curve"* checkbox to display both raw and normalized curves for each fluorescence channel.
  - Activate *"Message"* checkbox to display any pre-defined message for the run.
- *"Result"* column will display the test result of the samples for the run.
- *"Validity"* column will display the validity of the sample for the run.
- *"Fluorescence channels Ct"* column will display the Ct value for the samples.
- Click *"Preview Report"* to preview the report.

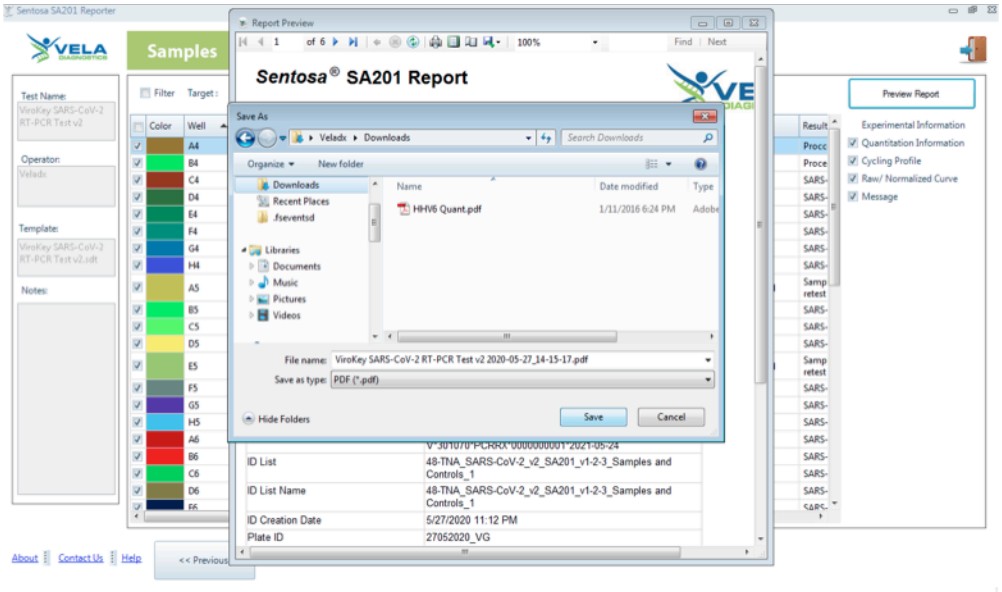


7.2. The *"Report Preview"* pop-up window will be displayed. The report can be saved, printed and / or exported to PDF or Excel.



7.3. *Click the "*  *" icon and select "PDF".*

The *"Save As"* pop-up window will be displayed. Select a location and click *"Save"* to save the report.



After the run is completed, proceed to *"Result interpretation"* on page 12.

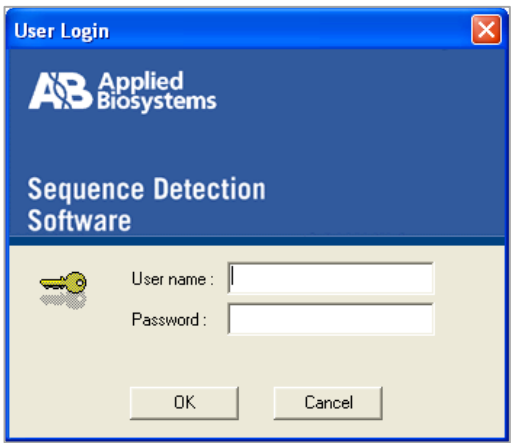
RT-PCR and manual data analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System

8. RT-PCR and manual data analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System

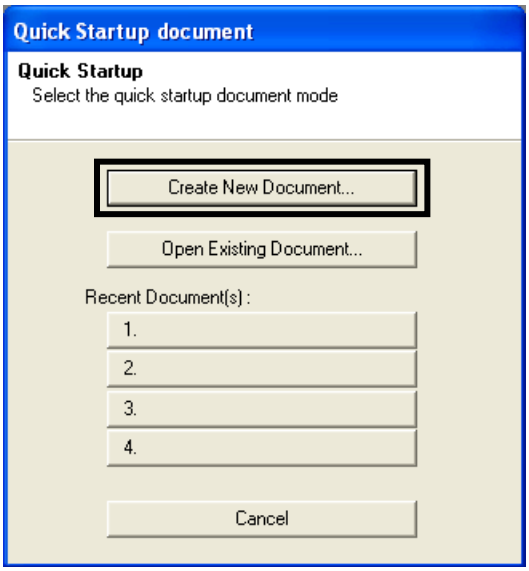
8.1. Turn on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System by pressing the power button on the instrument, and wait for the initiation procedure to be completed.
NOTE: Ensure the green indicator is lit and not flashing.

8.2. Run Reverse Transcription PCR

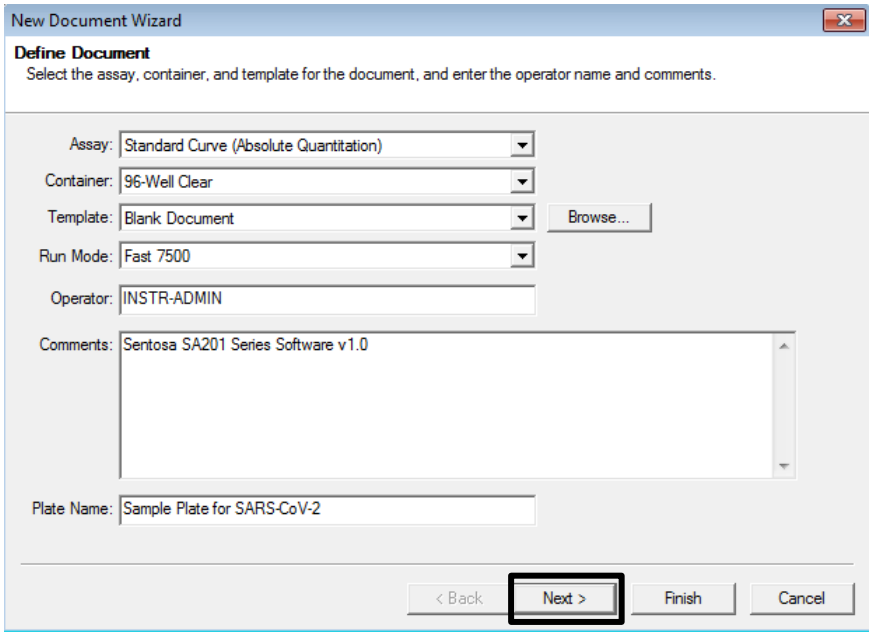
8.2.1. On the instrument's computer, launch the ABI 7500 Fast System SDS Software by double-clicking the  icon. The *"Login"* window opens, type the account name and password, and then click on *"OK"*.



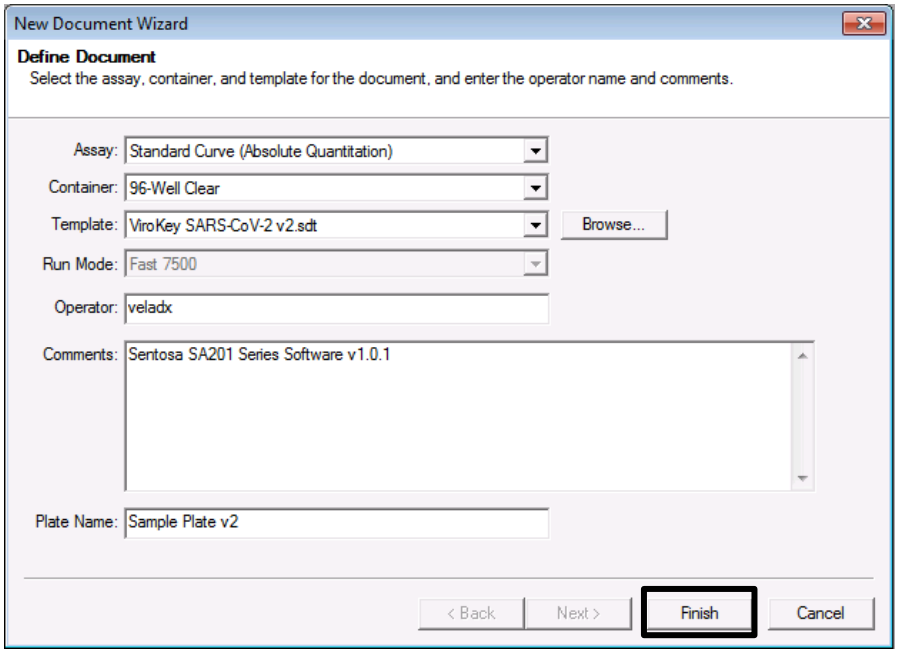
8.2.2. In the *"Quick Startup document"* dialog box, select *"Create New Document"*.



8.2.3. A *"New Document Wizard"* window appears for *"Define Document"*. In the *"Assay"* drop-down list, select *"Standard Curve (Absolute Quantitation)"*. The default setting for "*Container*" should be "*96-Well Clear*". The default setting for *"Template"* should be *"Blank Document"* for the first run. In the "*Run Mode*" drop-down list, select *"Fast 7500"*. Enter the name of the operator and the plate in the *"Operator"* and "*Plate Name"* fields respectively. Click *"Next"*.



If a template was created before, for *"Template"* select *"ViroKey SARS-CoV-2 v2".* In the *"Run Mode"* drop-down list, select *"Standard 7500".* Enter the name of the operator and the plate in the *"Operator"* and *"Plate Name"* fields respectively. Click *"Finish"*.

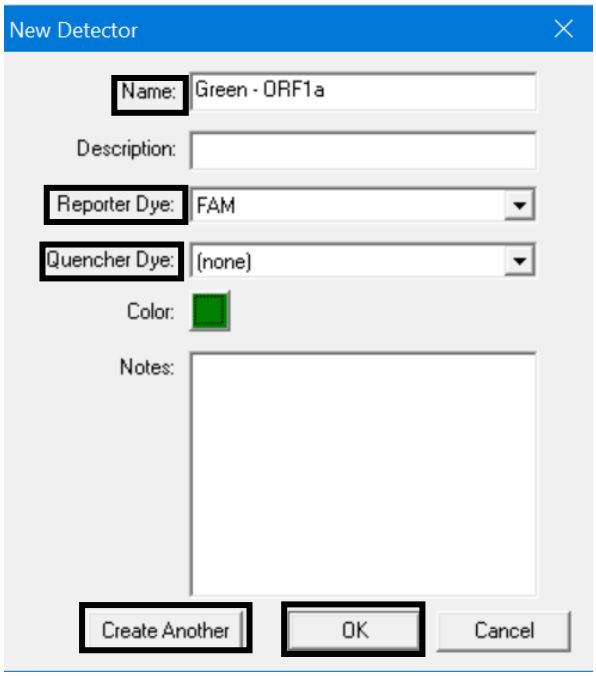


8.2.4. A "New Document Wizard" window appears for "Select Detectors". Click "New Detector" to create new "Green – ORF1a", "Orange – N" and "Red - EC" detectors, if needed, according to the table below:

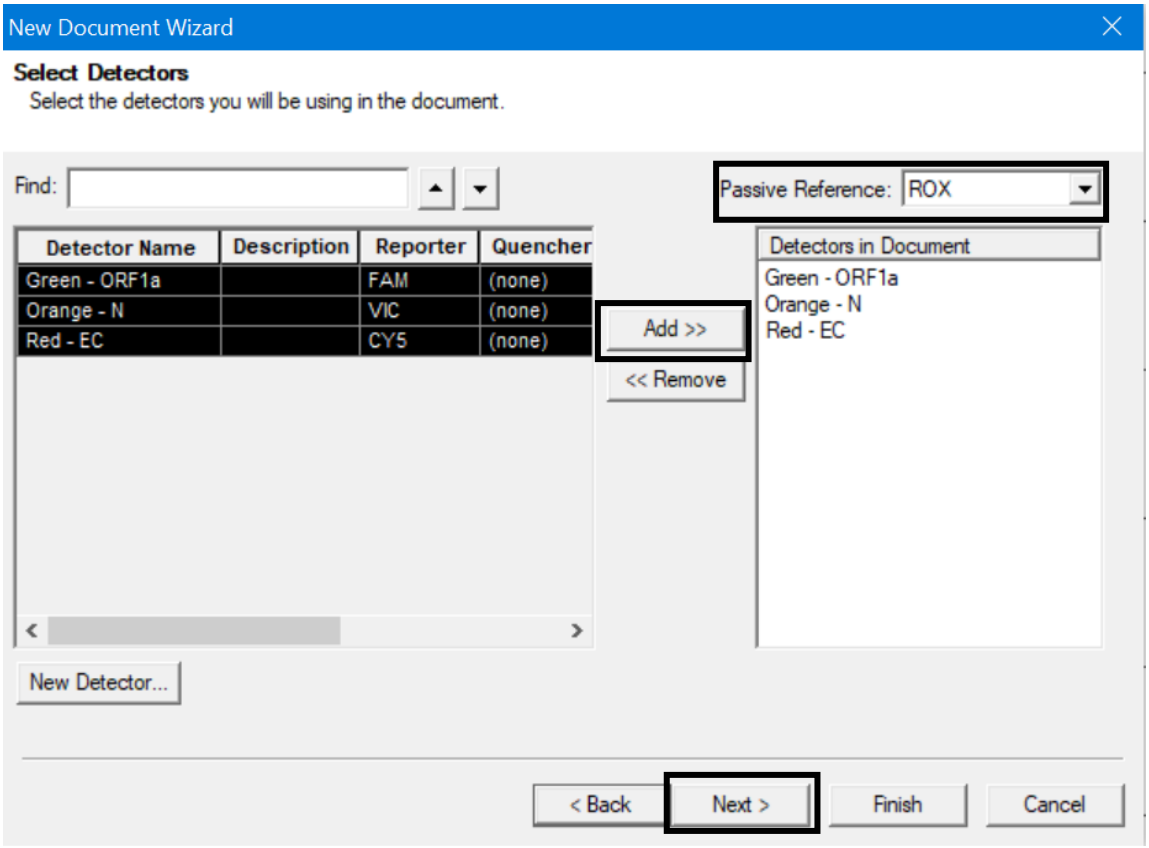
Detector Name	Reporter Dye	Quencher Dye
Green – ORF1a	FAM	(none)
Orange – N	VIC*	(none)
Red – EC	CY5	(none)

*: VIC Reporter Dye is used in place of HEX Reporter Dye due to similar excitation and emission wavelength, and the lack of HEX Reporter Dye in the ABI7500 list.

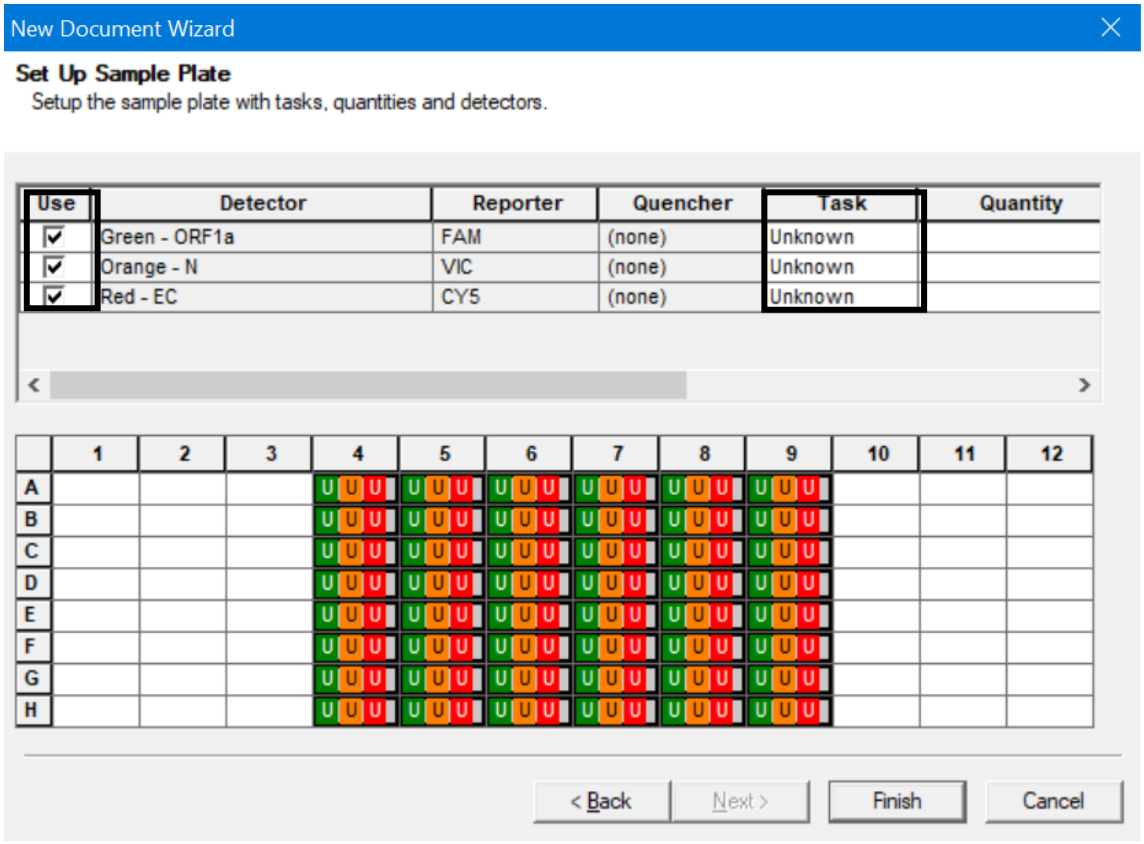
Select the *"Reporter Dye"* from the drop-down list. Select the *"Quencher Dye"* as *"None"*. Select the *"Color"* to match the detector. Click *"Create Another"* to specify new detector *"Name"*, *"Reporter Dye"* and *"Quencher Dye"*. Click *"OK"* after all detectors are created.



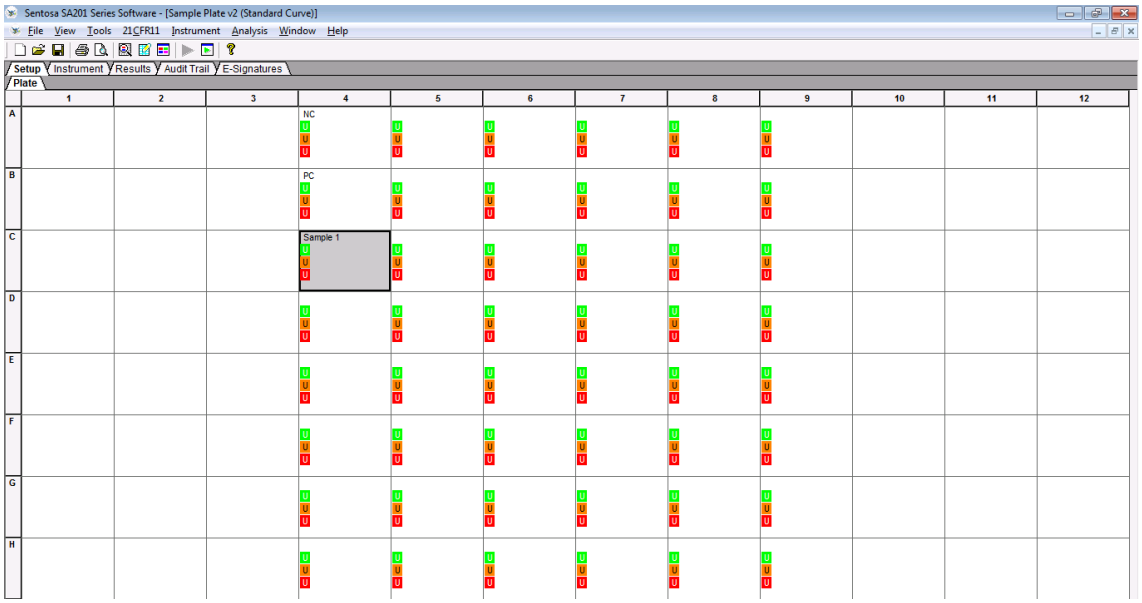
8.2.5. The *"New Document Wizard"* window for *"Select Detectors*" appears. Select the detectors and click *"Add"*. In the *"Passive Reference"* drop-down list, select *"ROX"*. Click *"Next"*.



8.2.6. A *"New Document Wizard"* window for *"Set Up Sample Plate"* appears. Select wells *"A4"* to *"H9"* (all wells from columns 4 to 9) and check the *"Use"* boxes for the detectors. The default setting for *"Task*" should be *"Unknown"*. Click *"Finish"*. The SDS Software will create the plate document.



8.2.7. A window for plate document appears. In the window, select *"Setup"* tab, and the *"Plate"* tab should be displayed. Name the wells of the plate by selecting them, then click on *"View"* menu, followed by *"Well Inspector"*.

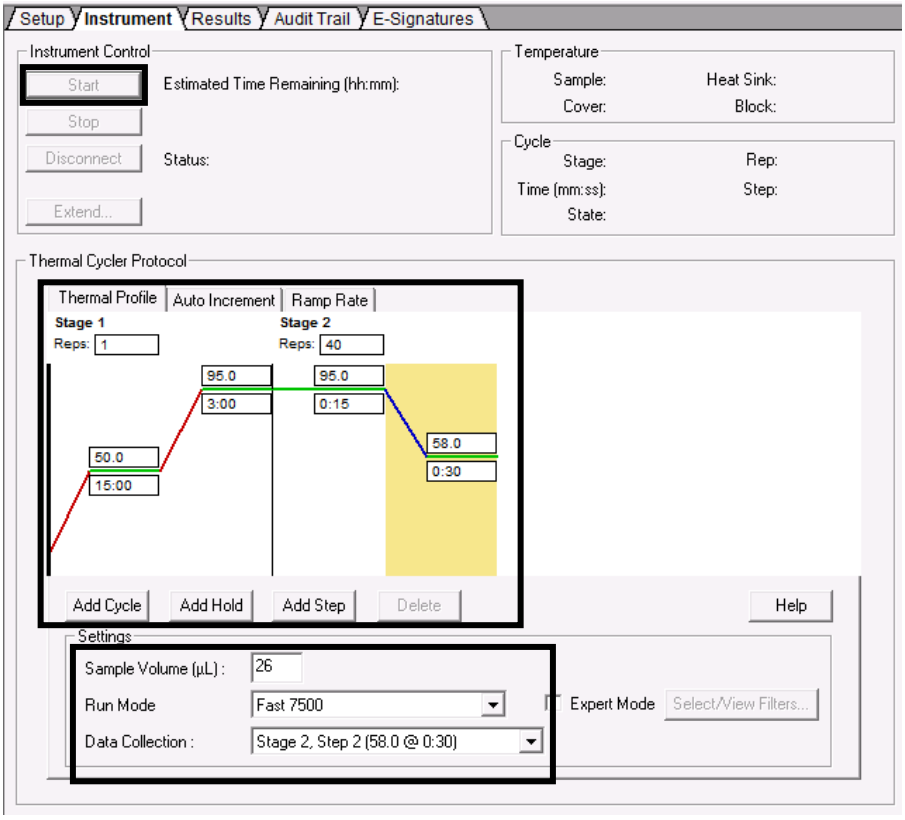


Enter the sample name by typing or barcode entry for each and verify the information of the well(s).

8.2.8. The thermal cycling conditions need to be manually specified according to the table below.

Stage	Reps	Temperature (°C)	Duration (min)
1	1	50	15:00
		95	3:00
2	40	95	0:15
		58	0:30

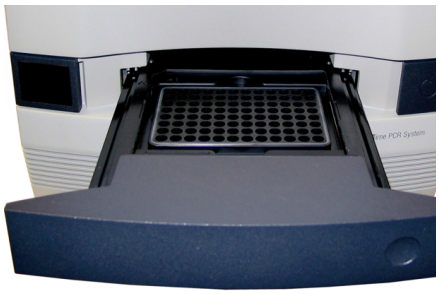
Under the *"Instrument"* tab, enter the thermal cycling condition values in the boxes. Accept the default settings for *"Auto Increment"* and *"Ramp Rate"* for all stages.

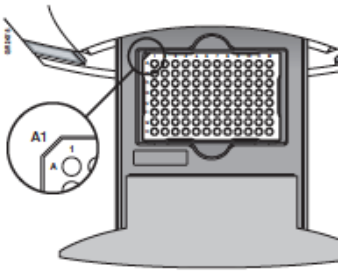


Specify the *"Sample Volume"* as 26 µL. The default setting for *"Run Mode"* should be *"Fast 7500"*. In the *"Data Collection"* drop-down list, select *"Stage 2, Step 2 (58.0 @ 0:30)"*.

8.2.9. Save the run file in *"sds"* format. The plate document can also be saved as a template document for future use. To do this, save the template file in *"sdt"* format in a preferred folder.

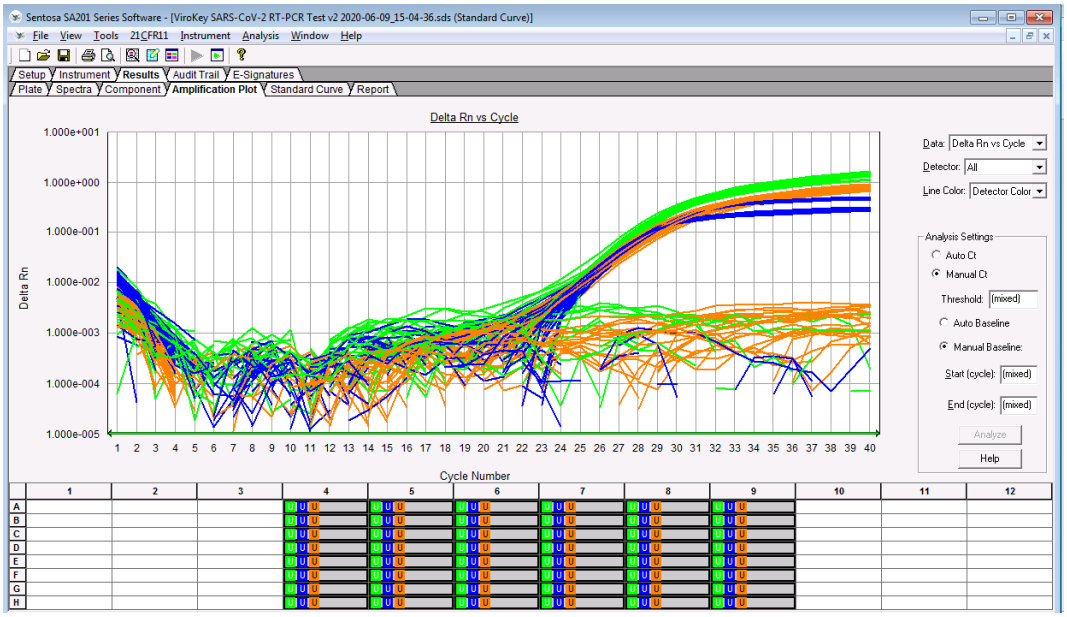
8.2.10. At the front of the Applied Biosystems® 7500 Fast Dx Real-Time PCR System, push to open the plate holder tray and load the plate into the precision plate holder. Ensure that the plate is properly aligned in the holder.



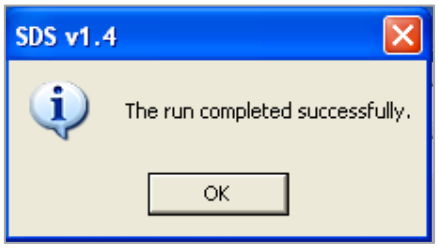


8.2.11. Under *"Instrument Control"* on the *"Instrument"* tab, click *"Start"*.

8.2.12. Information on real-time status of the run based on fluorescence emissions is displayed in the *"Results"* tab, under *"Amplification Plot"*.



8.2.13. At the end of the run, a message appears indicating whether or not the run is successful. All data generated is saved in the *".sds"* file specified in step 8.2.9.



8.2.14. After the run is completed, proceed to section 9, *"Data analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System"*.

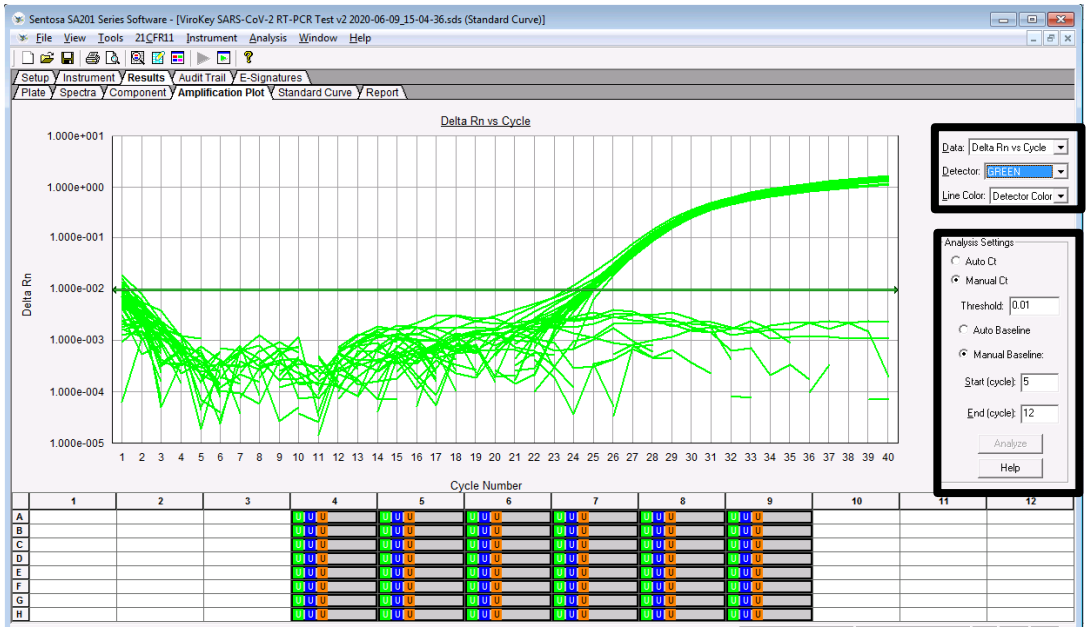
9. *Manual data analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System*

For the automated workflow runs, please refer to the plate layout below and analyze the results accordingly.

A				NC	S7	S15	S23	S31	S39		
B				PC	S8	S16	S24	S32	S40		
C				S1	S9	S17	S25	S33	S41		
D				S2	S10	S18	S26	S34	S42		
E				S3	S11	S19	S27	S35	S43		
F				S4	S12	S20	S28	S36	S44		
G				S5	S13	S21	S29	S37	S45		
H				S6	S14	S22	S30	S38	S46		

NC – Negative control, PC – Positive control, S – Sample

9.1. In the run window select the *"Results"* tab, followed by the *"Amplification Plot"* tab. Select *"Delta Rn vs Cycle"* in the *"Data"* drop-down list. In the *"Detector"* drop-down list, select a detector *("Green – ORF1a"*, *"Orange – N"*, or *"Red – EC"*).



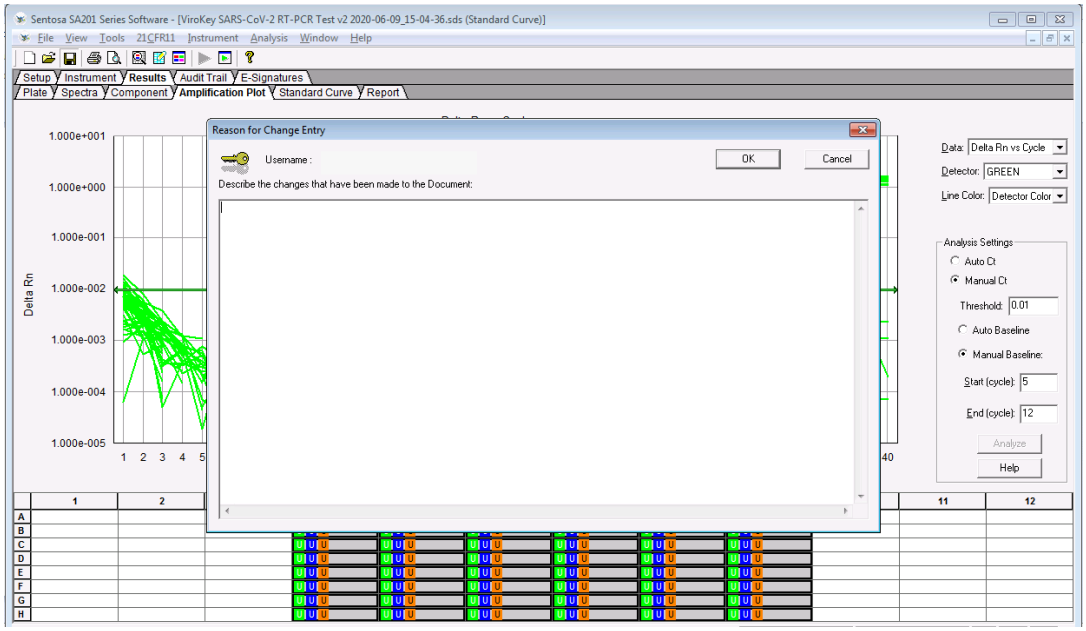
Set the threshold and baseline for the detectors according to the table below:

Detector	Threshold	Baseline	
		Start (cycle)	End (cycle)
Green – *ORF1a*	0.01	5	12
Orange – *N*	0.007	5	12
Red – EC	0.01	5	12

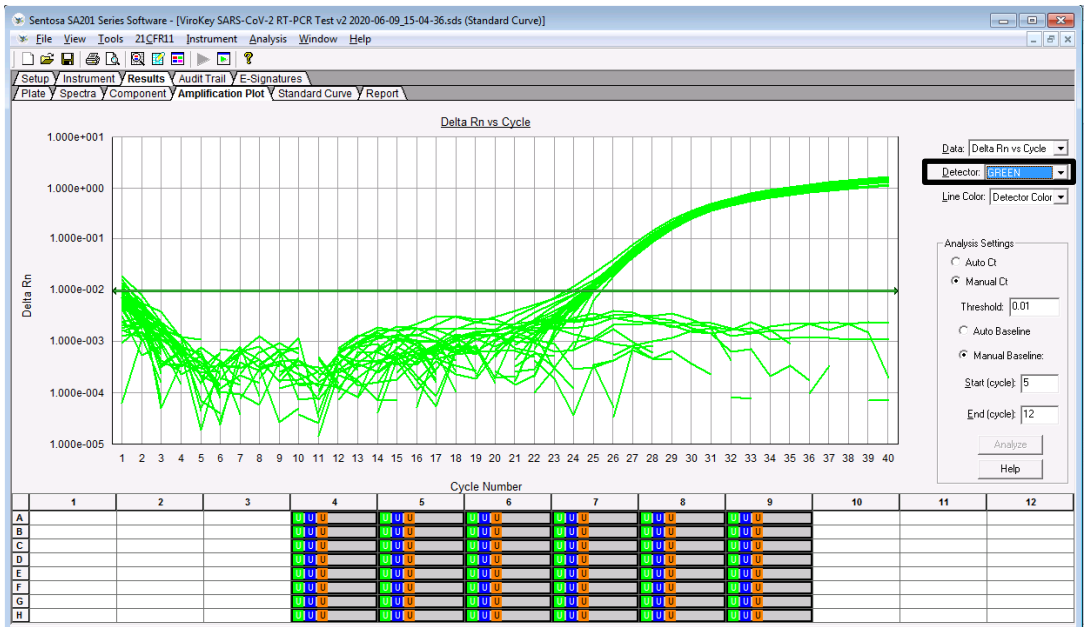
To set the threshold for the detector, select *"Manual Ct"* under *"Analysis Settings"* and enter the value. To set the baseline for the detector, select *"Manual Baseline"* under *"Analysis Settings"* and enter the values. Click *"Analyze"*. After which, the red threshold line turns green.

Repeat these steps to set the threshold and baseline values for all detectors.

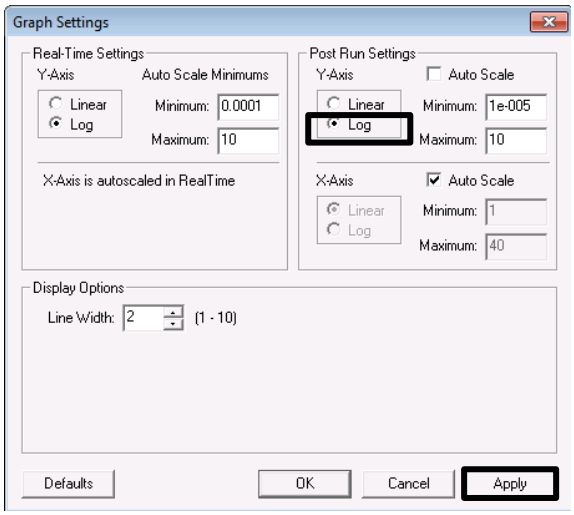
9.2. Select *"File"* from the main menu and *"Save"* from the drop-down list. *"Reason for Change Entry"* window will be displayed. Describe the changes made in the document or simply enter *"Save file"* or any other suitable text in the textbox and click *"OK"* to save the run document (*".sds"*) file.



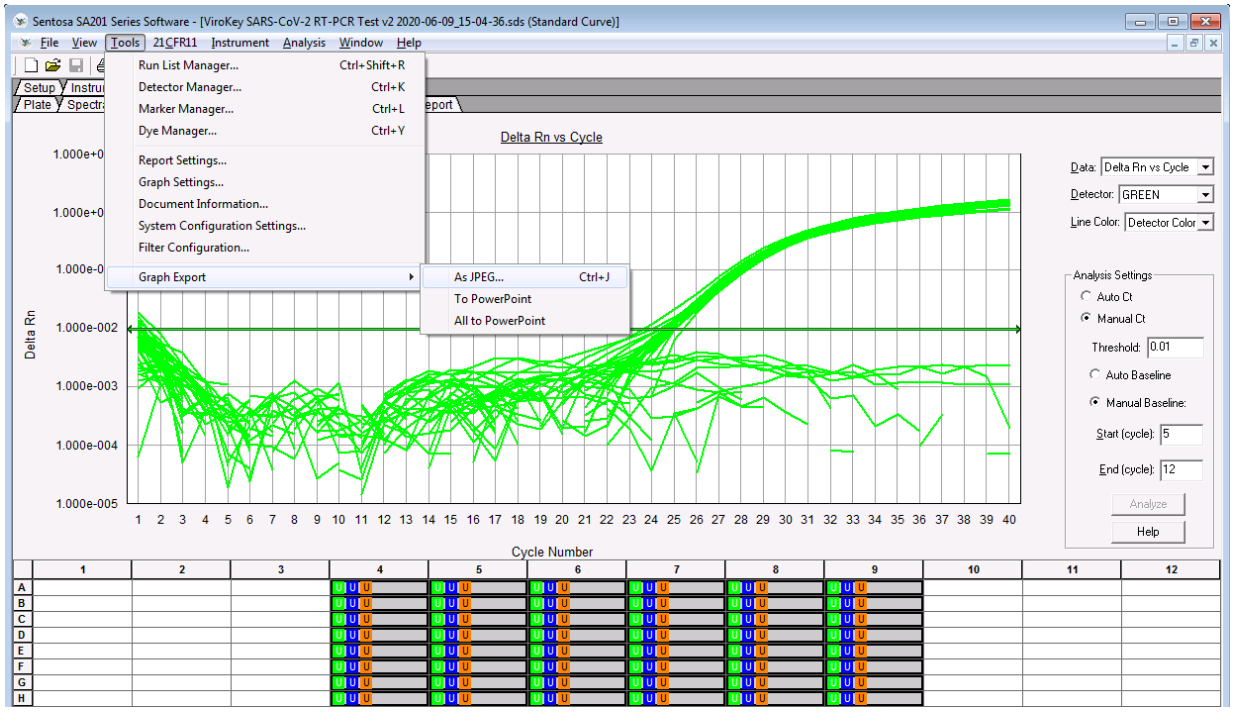
9.3. To generate graphs for specific samples or target channel, select the corresponding sample wells at the bottom of the window, then choose the desired target channel from the "Detector" drop-down list. Select *"GREEN"* for the analysis of the SARS-CoV-2 target *ORF1a*, *"ORANGE"* for the analysis of the SARS-CoV-2 target *N*, and *"RED"* for the analysis of the extraction control.



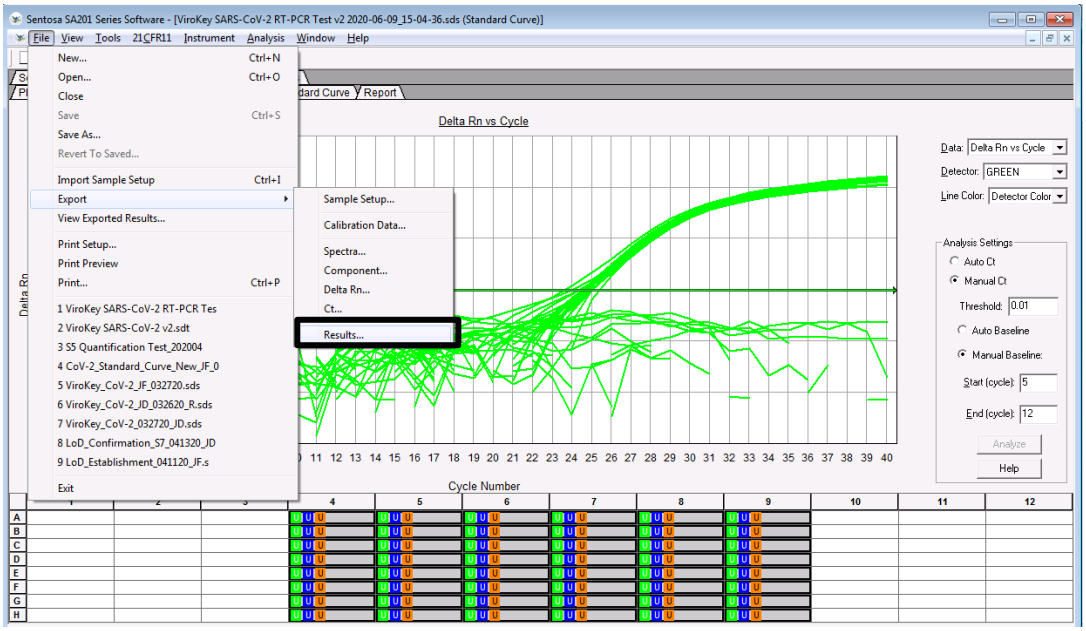
Right click on the graph to select *"Graph setting"*. In *"Post Run setting"* category, choose *"Log"* for Y-axis. Click *"Apply"*.



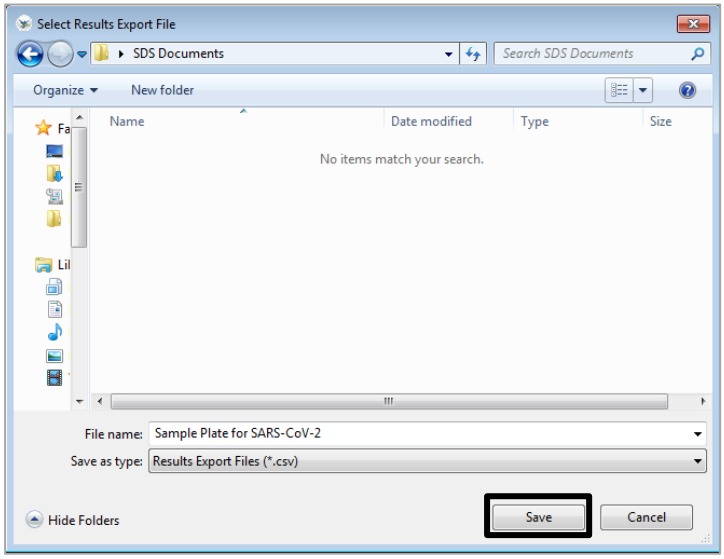
9.4. To export the graphs for the run, select *"Tools"* menu -> *"Graph Export"*, then choose the desired format to export the graph in.



9.5. To export results, save the ".*sds*" file first. Select "*File*" menu -> "*Export*", then choose the desired information to export.



9.6. The *"Select Results Export"* pop-up window appears. Accept the default setting for *"Save as type"*. Navigate to the desired folder and enter the name of the result export file (*".csv"*). Click *"Save"*.



After the run is completed, proceed to *"Result interpretation"* on page 12.

Instrument maintenance

After every run, discard used sample tubes, plates, reagents and tips according to the local safety regulations. All samples and waste should be considered potentially infectious.

A reservoir collects liquid waste generated during the nucleic acid extraction procedure. Dispose the liquid waste according to the local safety and environment regulations. Dispose the biohazard bags after each run.

Perform regular cleaning of the instruments *Sentosa*® SX101, Thermo Fisher™ KingFisher™ Flex, Hamilton Microlab® STAR™, and *Sentosa*® SA201 after each run. Refer to the respective instrument user manuals or maintenance videos for detailed procedures.

Ensure that maintenance is performed regularly to minimize the risk of error.

Always wear the appropriate personal protective equipment (PPE: lab coat, gloves, goggles, mask) during cleaning / maintenance procedures.

Troubleshooting guide

The troubleshooting guide may be helpful in solving any problems that may arise. For more information, please contact the authorized Vela Diagnostics representative. Vela Diagnostics Service and Support is always ready to answer any questions about the information and protocols in this user manual or sample and assay technologies (for contact information, refer to the back cover).

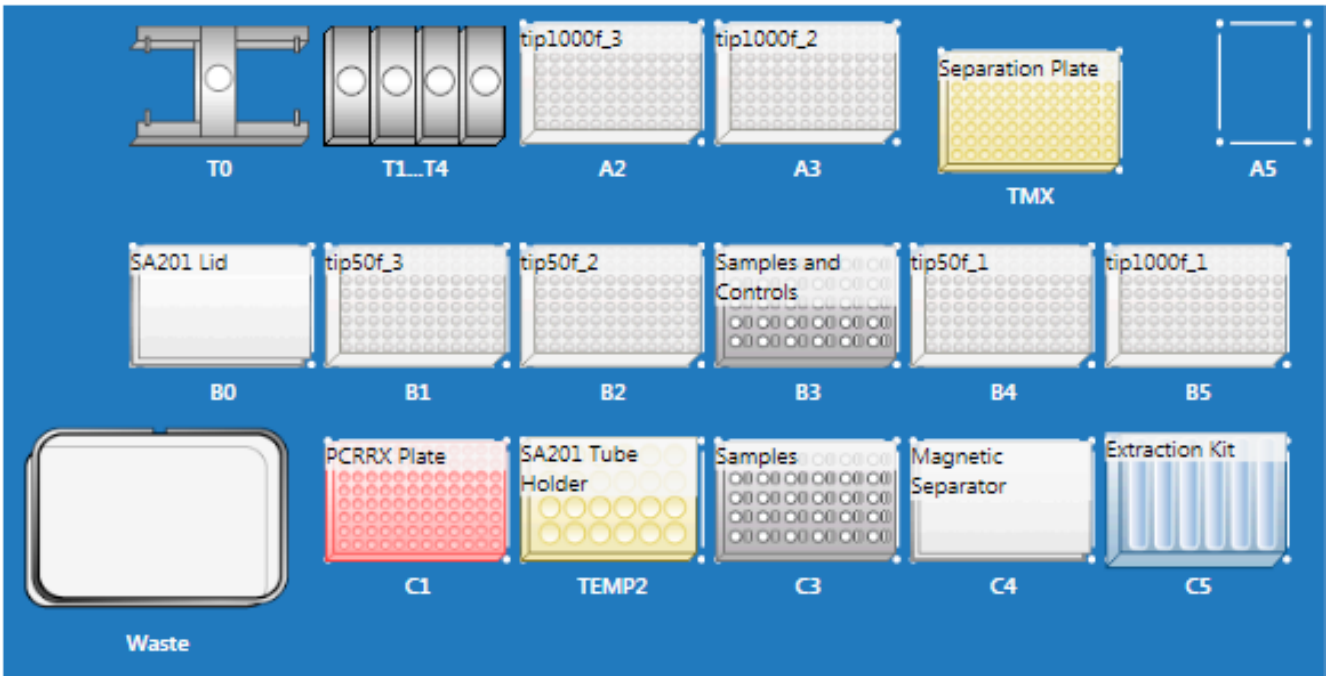
		Comments and recommended actions
1.	**General handling**	
a)	Error message displayed on the screen	When an error message is displayed during a protocol run, please refer to the instrument user manuals.
2.	**Precipitates in the reagents of the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) / ViroKey® HT Virus Total Nucleic Acid Kit (4x96)**	
a)	Storage of reagents	Reagents might precipitate upon storage. If required, shake vigorously to dissolve the precipitates.
3.	**Consistent high Ct values observed for samples**	
a)	Magnetic beads were not completely re-suspended	Mag (magnetic beads) requires thorough vortexing before use to ensure proper resuspension.
b)	Frozen samples were not mixed properly after thawing	Thaw frozen samples with mild agitation to ensure thorough mixing.
c)	Degraded nucleic acids	Ensure that samples are stored correctly and not subjected to multiple freeze-thaw cycles. Repeat the extraction procedure with new samples.
d)	Incomplete sample lysis	Ensure that Buffer D1 (lysis buffer) does not contain precipitates. If required, shake vigorously to dissolve the precipitates.
e)	Clogging of pipette tip due to insoluble material in the samples	Insoluble material was not removed from the sample prior to starting the extraction procedure on the *Sentosa*® SX101 instrument. To remove insoluble material, centrifuge the diluted sample suspension at 3,000 x *g* for 1 minute, and transfer the supernatant to a fresh sample tube.
4.	**No signal with positive control (PC) in the Green, Orange or Red fluorescence channels**	
a)	PCR conditions do	Ensure that the correct thermal cycling conditions are

		Comments and recommended actions
	not comply with the protocol	input into the *Sentosa*® SA201 Series Software or ABI 7500 Fast System SDS Software.
b)	Incorrect PCR configuration	Ensure that the correct thermal cycling conditions are input into the *Sentosa*® SA201 Series Software Software or ABI 7500 Fast System SDS Software.
c)	Storage conditions for one or more components did not comply with the instructions given in the "Storage" section	Check the storage condition (refer to the kit label) of the reagents and use a new kit, if necessary.
d)	Extraction / assay kit has expired	Check the expiration date (refer to the kit label) of the reagents and use a new kit, if necessary.
e)	Incorrect passive reference setting	Check the passive reference setting is set correctly to ROX in the well inspector and reanalyze.
5.	**Weak or no signal of the extraction control (EC) in the Red fluorescence channel subjected to extraction using the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) / ViroKey® HT Virus Total Nucleic Acid Kit (4x96)**	
a)	PCR conditions do not comply with the protocol	Ensure that the correct thermal cycling conditions are input into the *Sentosa*® SA201 Series Software Software or ABI 7500 Fast System SDS Software. For PCR configuration, please refer to section 4 of the troubleshooting guide.
b)	PCR inhibition	Dilute the extraction eluent 10 times and repeat the PCR. Collect new sample and repeat the workflow.
c)	EC is not added into the sample	Ensure that EC was added during the sample pre-treatment and lysis steps.
d)	Loss of nucleic acid during extraction	No signal of the extraction control may indicate the loss of nucleic acid during the extraction. Ensure the correct SX101 application is used and follow the instructions closely. Refer to section 3 of the troubleshooting guide.
e)	Storage conditions for one or more kit components did not comply with the instructions given in the "Storage" section	Check the storage condition (refer to the kit label) of the reagents and use a new kit if necessary.

		Comments and recommended actions
f)	Extraction / assay kit has expired	Check the expiration date (refer to the kit label) of the reagents and use a new kit if necessary.
6.	**Signals with the negative control in the Green and Orange fluorescence channels of the analytical PCR**	
a)	Contamination occurred during extraction / PCR set-up	Repeat the extraction and PCR protocols with new reagents. Ensure that the workspace and instruments are decontaminated as recommended. First check the Rn for true amplification profile and rule out baseline issues.
b)	Baseline issue	Check the raw (Rn) signal of the green channel of the NC. Sudden jumps in baseline can become false positive call in the dRn.

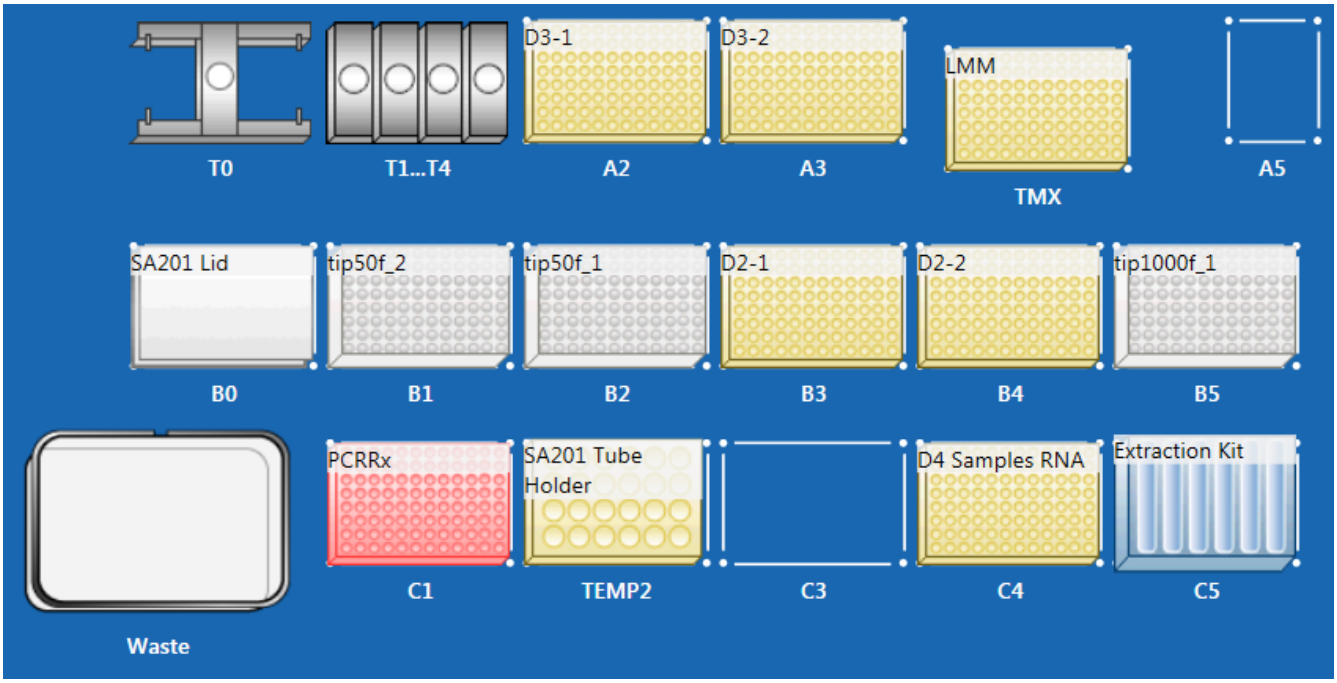
Appendix for ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48)

Appendix A: *Sentosa*® SX101 layout for 48-1 ViroKey SARS-CoV-2-v2 v3-3 application



Position	Labware
T0	*Sentosa*® SX Gripper
T1 to T4	*Sentosa*® SX Dispensing Tools
A2	*Sentosa*® SX Non-Partition 1000 µL Filter Tips
A3	*Sentosa*® SX Non-Partition 1000 µL Filter Tips
TMX	*Sentosa*® SX Deepwell Plate 96/2000 µL
A5	Empty
B0	SA201 Lid
B1	*Sentosa*® SX Non-Partition 50 µL Filter Tips
B2	*Sentosa*® SX Non-Partition 50 µL Filter Tips
B3 and C3	*Sentosa*® SX Rack 0.5 + Adaptor / 1.5 / 2.0 mL
B4	*Sentosa*® SX Non-Partition 50 µL Filter Tips
B5	*Sentosa*® SX Non-Partition 1000 µL Filter Tips
C1	MicroAmp® Fast Optical 96-Well Reaction Plate, 0.1 mL
TEMP2	*Sentosa*® SA201 Tube Holder Rack
C4	*Sentosa*® SX Magnetic Separator
C5	*Sentosa*® SX Reservoir Rack 7

Appendix B: *Sentosa*® SX101 layout for 96-1_ViroKey-KF SARS-CoV-2-v2_v3-2 application



Position	Labware
T0	*Sentosa*® SX Gripper
T1 to T4	*Sentosa*® SX Dispensing Tools
A2	KingFisher™ 96-Deepwell Plate
A3	KingFisher™ 96-Deepwell Plate
TMX	KingFisher™ 96-Deepwell Plate
A5	Empty
B0	SA201 Lid
B1	*Sentosa*® SX Non-Partition 50 µL Filter Tips
B2	*Sentosa*® SX Non-Partition 50 µL Filter Tips
B3	KingFisher™ 96-Deepwell Plate
B4	KingFisher™ 96-Deepwell Plate
B5	*Sentosa*® SX Non-Partition 1000 µL Filter Tips
C1	MicroAmp® Fast Optical 96-Well Reaction Plate, 0.1 mL
TEMP2	*Sentosa*® SA201 Tube Holder Rack
C3	Empty
C4	KingFisher™ 96-Deepwell Plate
C5	*Sentosa*® SX Reservoir Rack 7

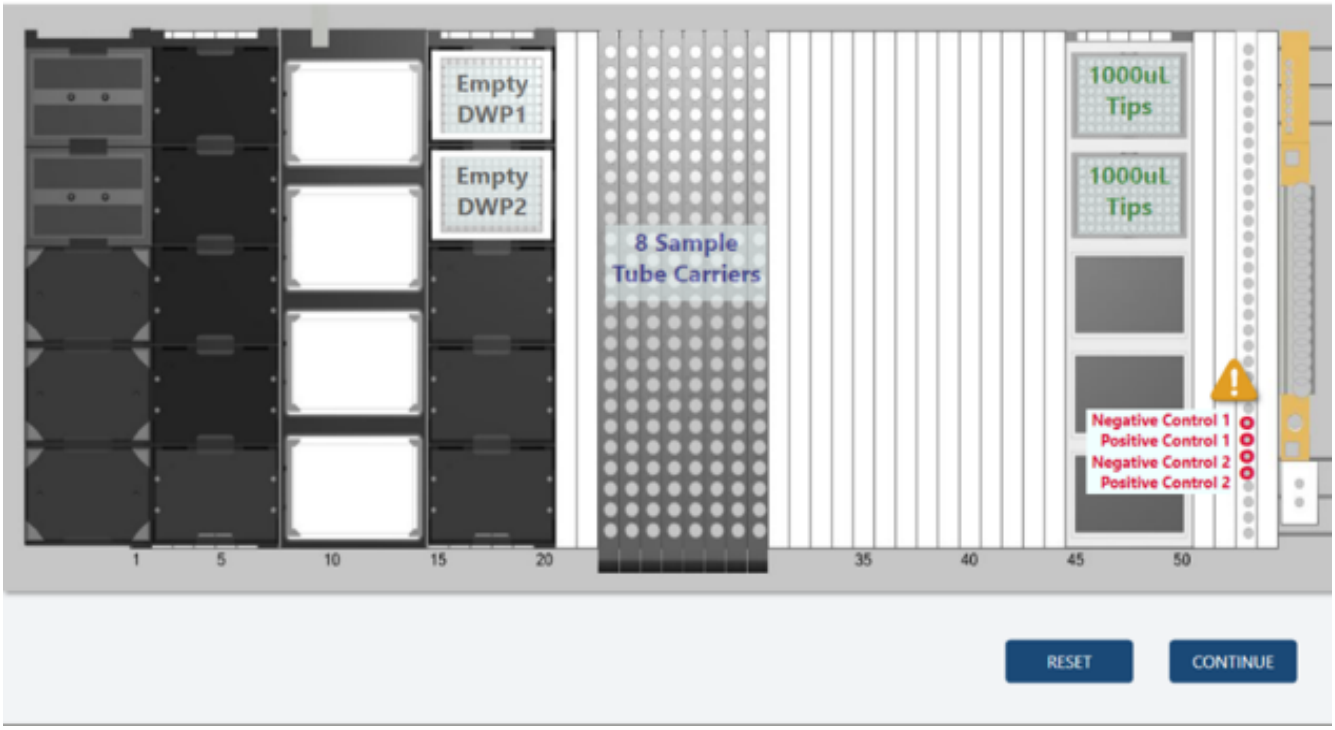
Appendix for ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96)

Appendix for Sample Plate Preparation

Appendix A: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 1 deepwell plate) using STAR8AL96 Vela_SampleTransfer_V1.2.med application

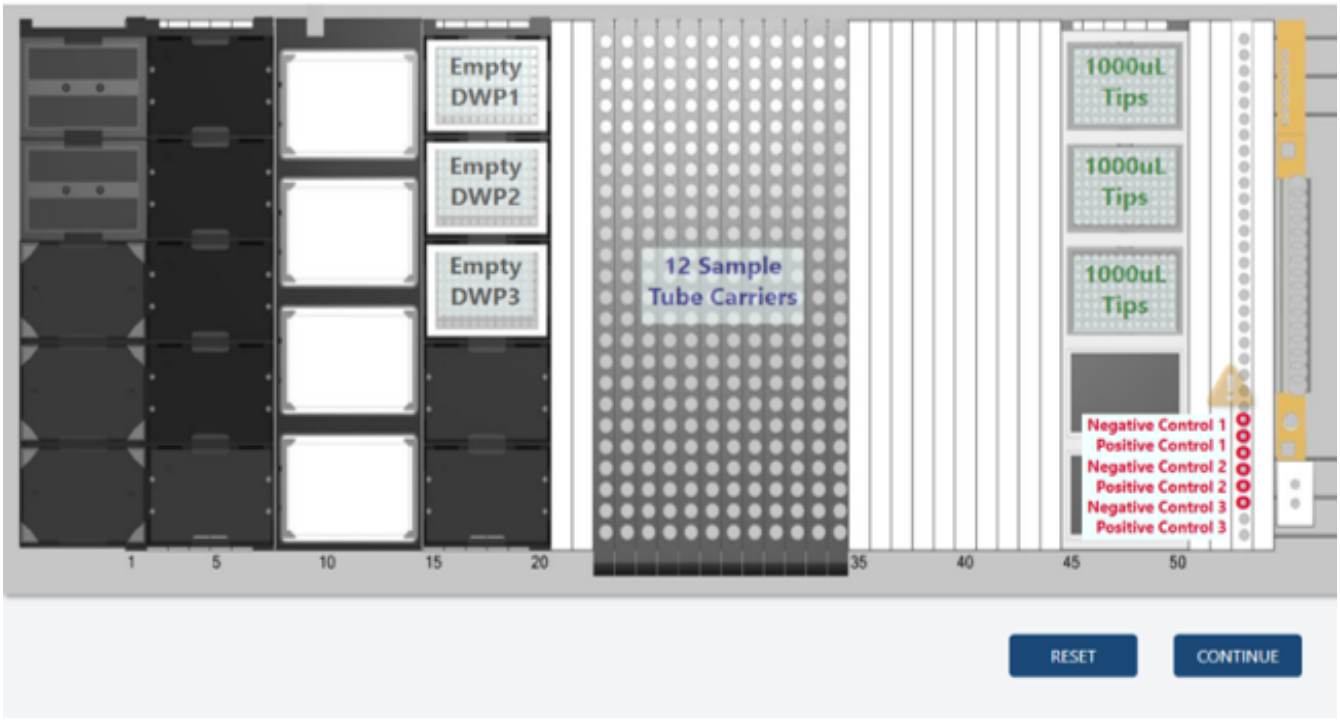
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 26	4 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (96)
53	NC & PC (Positions 25 and 26)

Appendix B: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 2 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application



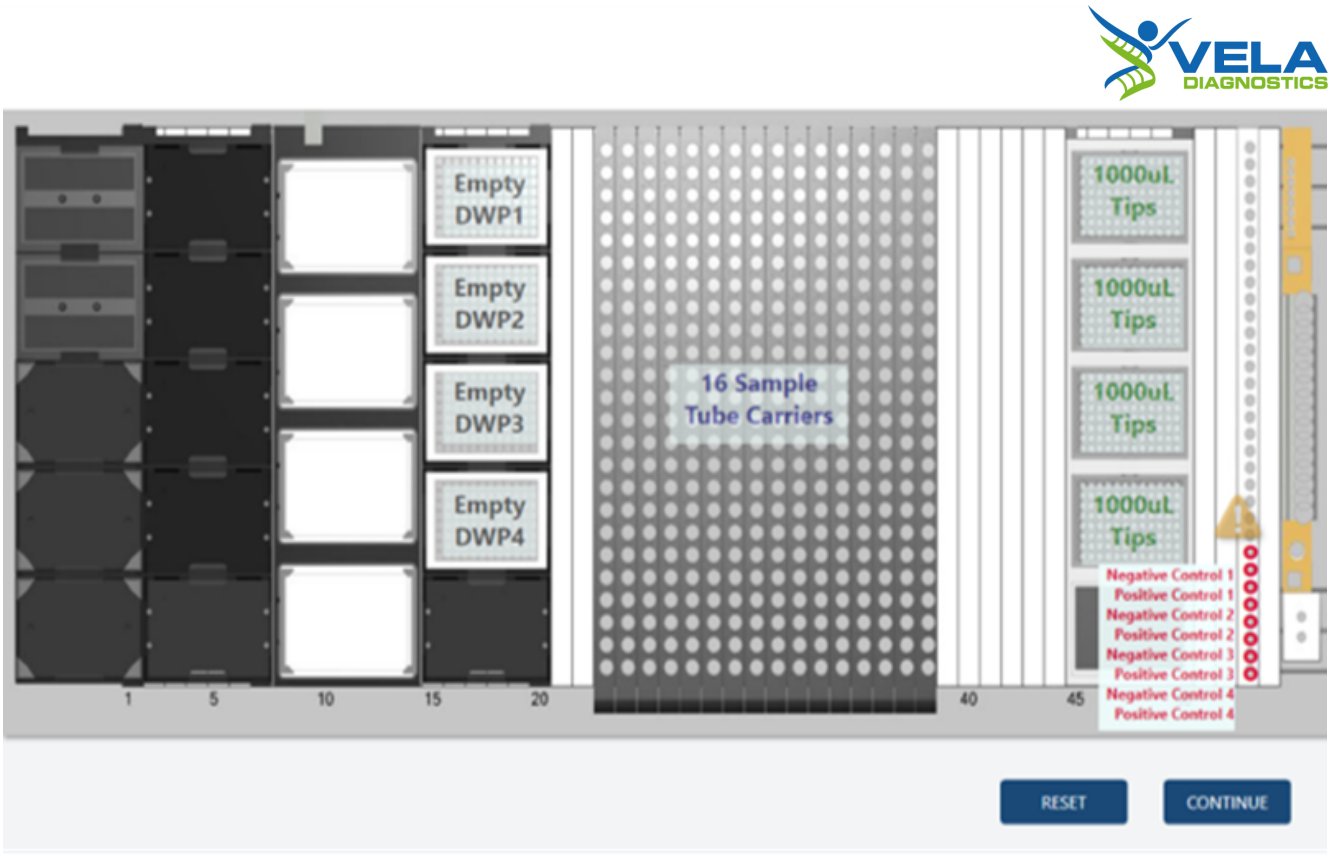
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	2x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 30	8 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (2x96)
53	2x NC & 2x PC (Positions 25 to 28)

Appendix C: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 3 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	3x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 34	12 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (3x96)
53	3x NC & 3x PC (Positions 25 to 30)

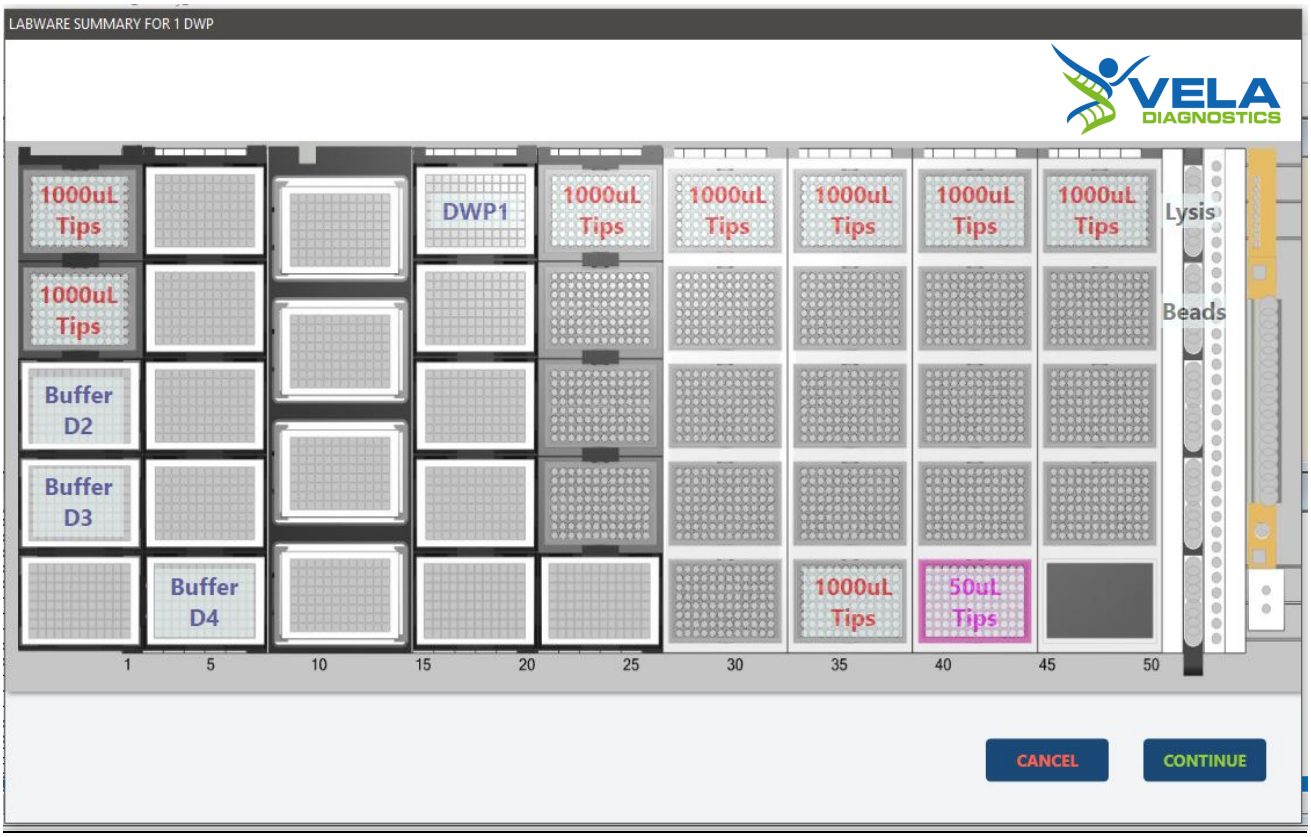
Appendix D: Layout of the Hamilton Microlab® STAR™ platform for sample plate preparation (with 4 deepwell plates) using STAR8AL96 Vela_SampleTransfer_V1.2.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	4x HT U Deepwell Plate, Barcoded, 2.2 mL
23 to 38	16 Sample Tube Carriers
45 to 50	HT Conductive 1 mL Filter Tips (4x96)
53	4x NC & 4x PC (Positions 25 to 32)

Appendix for Viral RNA Extraction

Appendix E: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 1 deepwell plate) using STAR8AL96 Vela_ViroKey_V3.10.med application

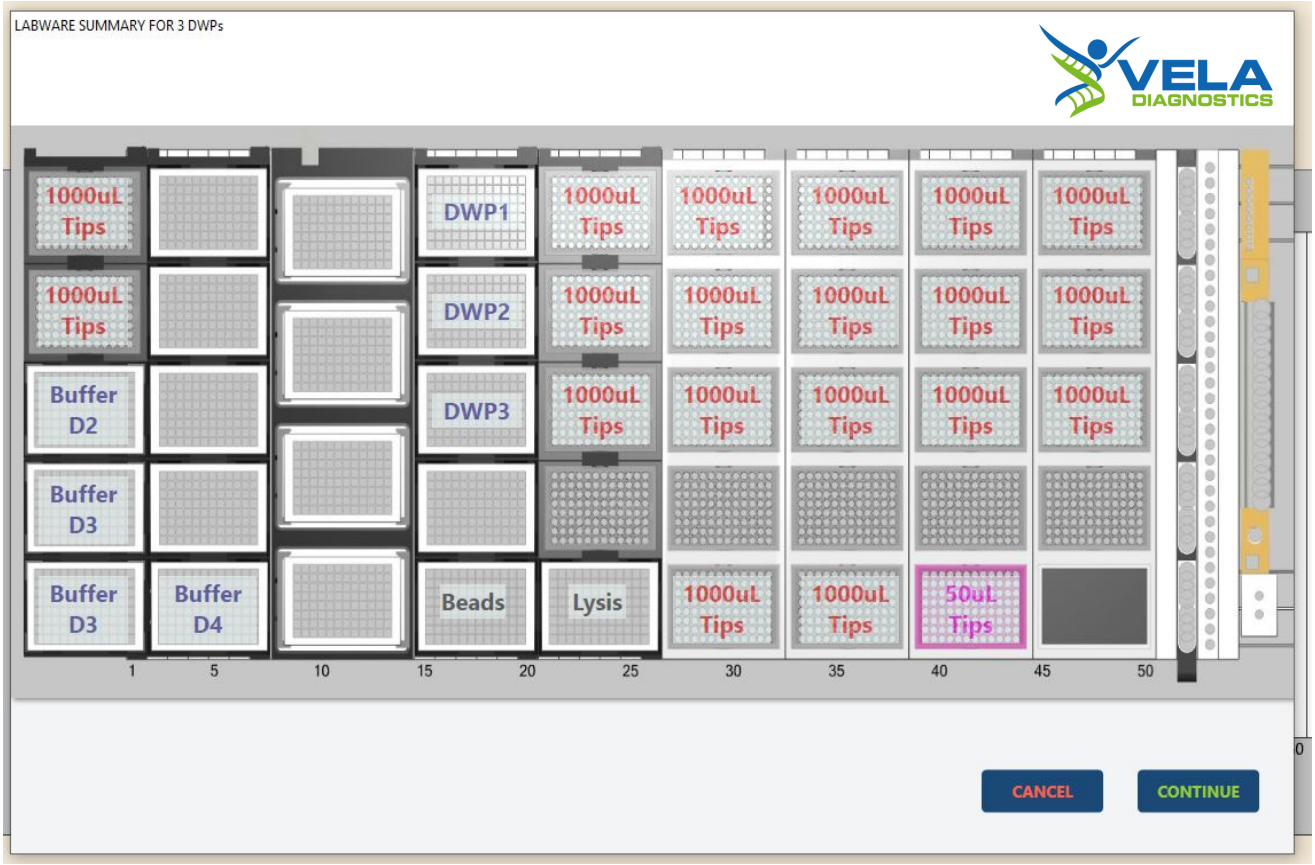


Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 2x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	HT U Deepwell Plate, Barcoded, 2.2 mL
21 to 26	HT Conductive 1 mL Filter Tips (96)
27 to 32	HT Conductive 1 mL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (2x96)
39 to 44	• HT Conductive 1 mL Filter Tips (96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (96)
52	2x HT Reagent Tub with Lid, 60 mL

Appendix F: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 2 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application

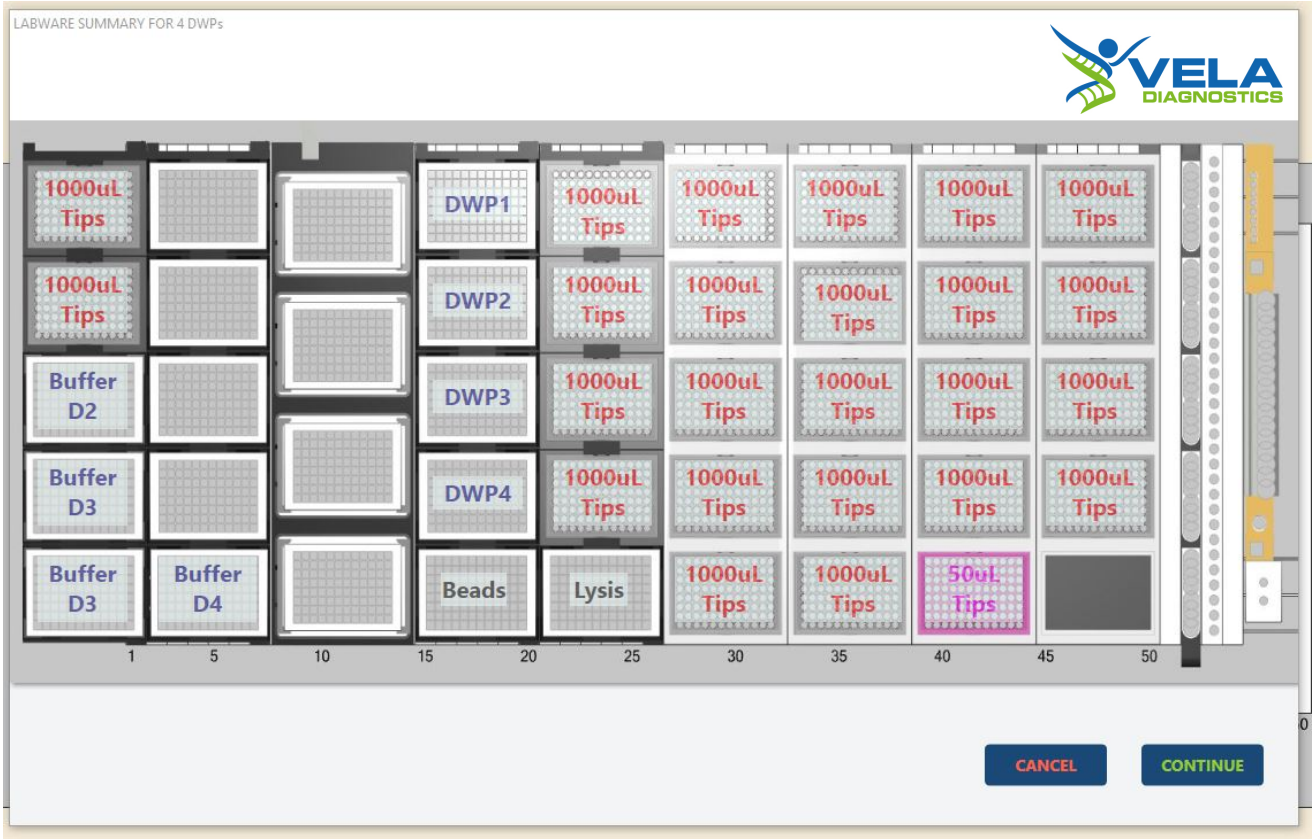
Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	• 2x HT U Deepwell Plate, Barcoded, 2.2 mL • HT 300 mL Reservoir
21 to 26	• HT Conductive 1 mL Filter Tips (2x96) • HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (3x96)
33 to 38	HT Conductive 1 mL Filter Tips (3x96)
39 to 44	• HT Conductive 1 mL Filter Tips (2x96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (2x96)

Appendix G: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 3 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application



Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	• 3x HT U Deepwell Plate, Barcoded, 2.2 mL • HT 300 mL Reservoir
21 to 26	• HT Conductive 1 mL Filter Tips (3x96) • HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (4x96)
33 to 38	HT Conductive 1 mL Filter Tips (4x96)
39 to 44	• HT Conductive 1 mL Filter Tips (3x96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (3x96)

Appendix H: Layout of the Hamilton Microlab® STAR™ platform for viral RNA extraction (with 4 deepwell plates) using STAR8AL96 Vela_ViroKey_V3.10.med application



Track(s)	Description
1	• HT Conductive 1 mL Filter Tips (2x96) • 3x HT 300 mL Reservoir
2 to 7	HT 300 mL Reservoir
8 to 14	Empty
15 to 20	• 4x HT U Deepwell Plate, Barcoded, 2.2 mL • HT 300 mL Reservoir
21 to 26	• HT Conductive 1 mL Filter Tips (4x96) • HT 300 mL Reservoir
27 to 32	HT Conductive 1 mL Filter Tips (5x96)
33 to 38	HT Conductive 1 mL Filter Tips (5x96)
39 to 44	• HT Conductive 1 mL Filter Tips (4x96) • HT Conductive 50 µL Filter Tips (96)
45 to 50	HT Conductive 1 mL Filter Tips (4x96)

Appendix I: Consumable summary for 1 deepwell plate

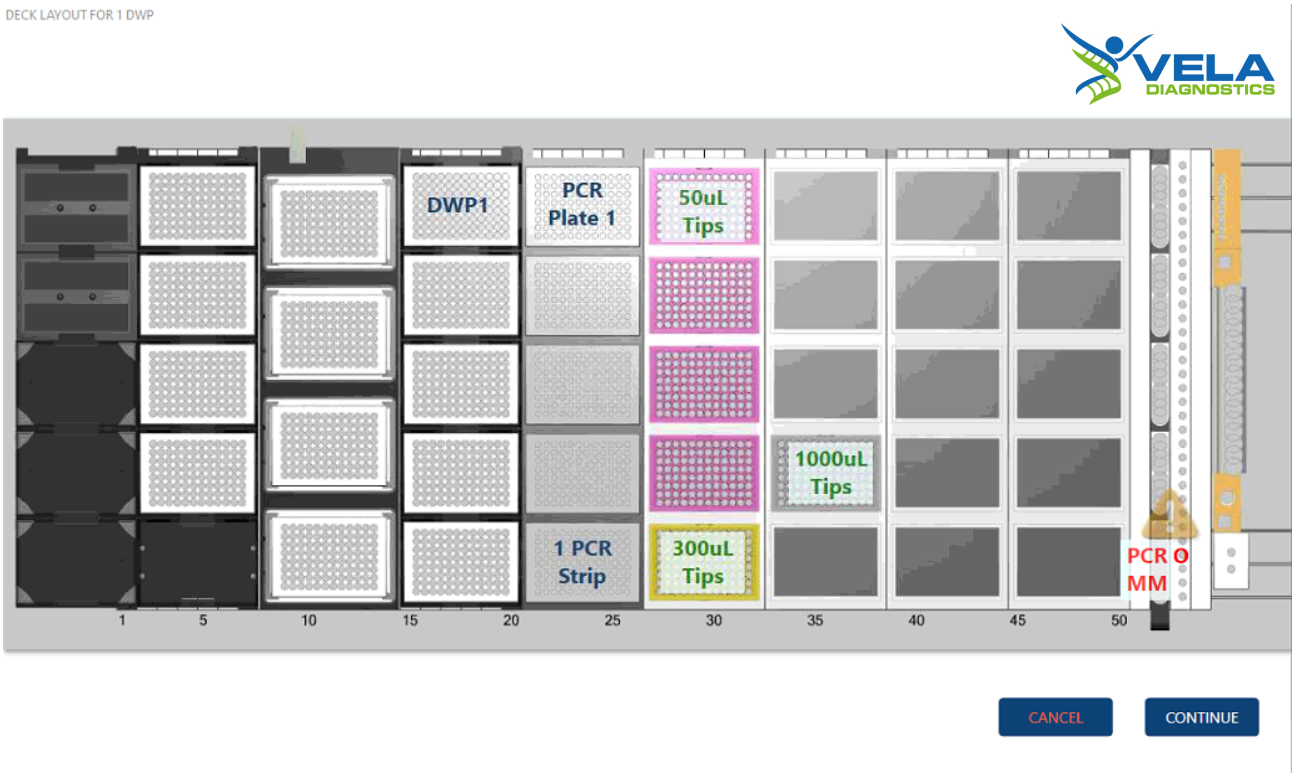
Appendix J: Consumable summary for 2 deepwell plates

Appendix K: Consumable summary for 3 deepwell plates

Appendix L: Consumable summary for 4 deepwell plates

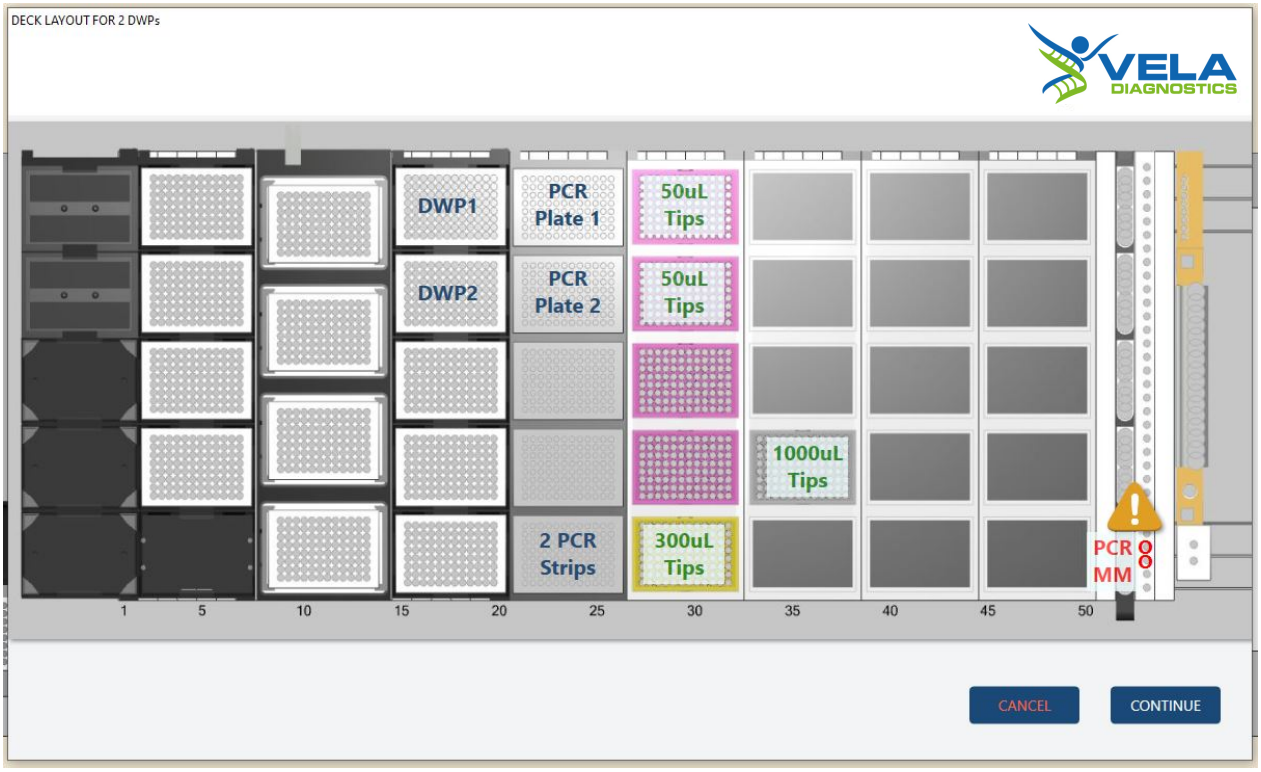
Appendix for HT PCR setup

Appendix M: Layout of the Hamilton Microlab® STAR™ platform for HT PCR setup (with 1 sample plate) using STAR8AL96 Vela_PCRsetup_V1.3.med application



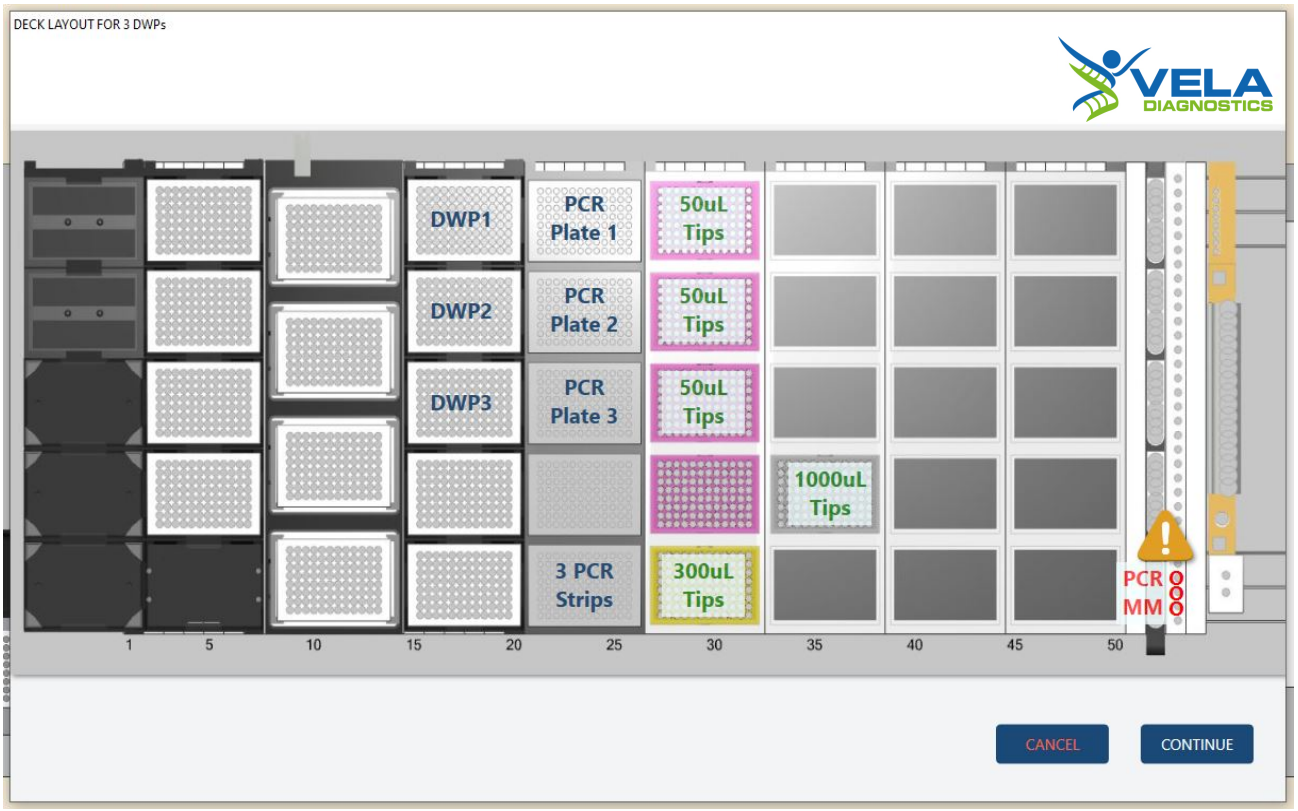
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• HT U Deepwell Plate, Barcoded, 2.2 mL
21 to 26	• MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 1x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	PCR MM (Position 29)

Appendix N: Layout of the Hamilton Microlab® STAR™ platform for HT PCR setup (with 2 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application



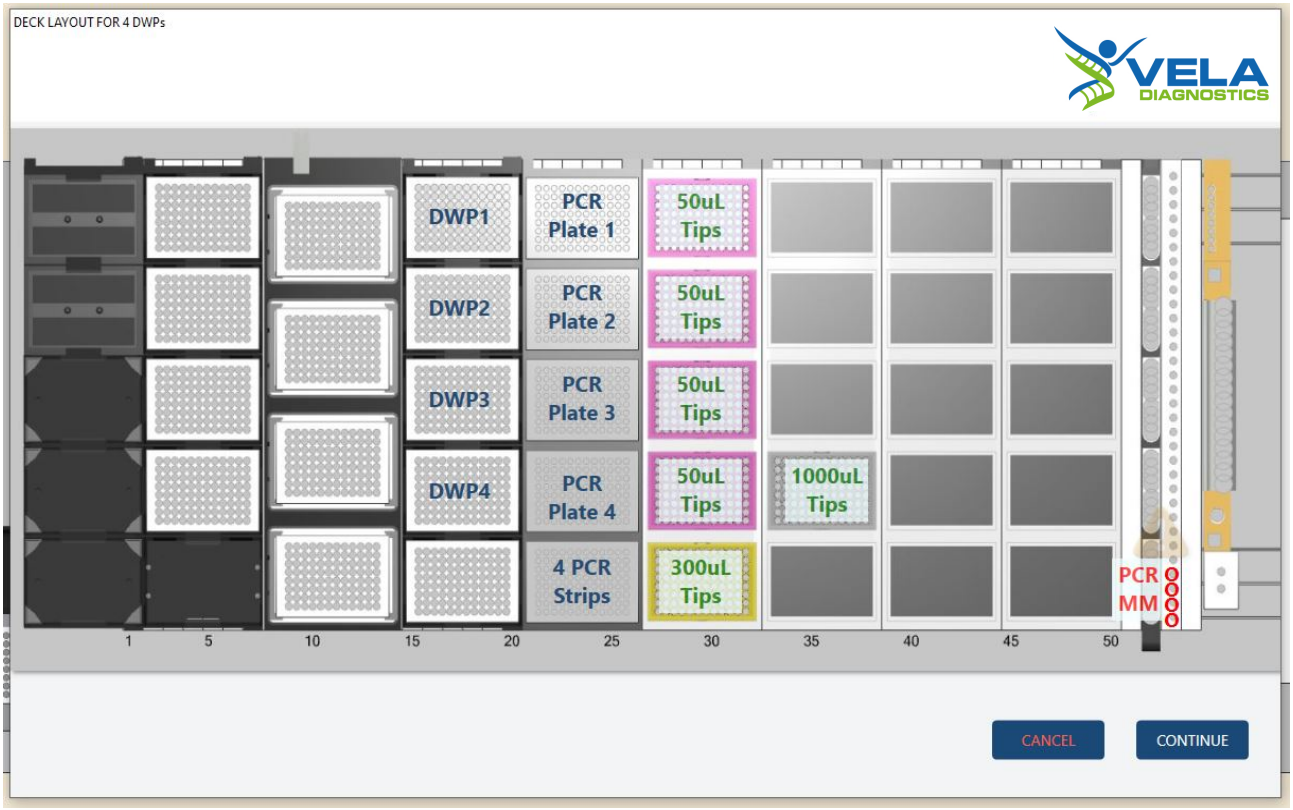
Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• 2x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	• 2x MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 2x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (2x96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	2x PCR MM (Positions 29 and 30)

Appendix O: Layout of the Hamilton Microlab® STAR platform for HT PCR setup (with 3 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• 3x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	• 3x MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 3x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (3x96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	3x PCR MM (Positions 29 to 31)

Appendix P: Layout of the Hamilton Microlab® STAR™ platform for HT PCR setup (with 4 sample plates) using STAR8AL96 Vela_PCRsetup_V1.3.med application



Track(s)	Description
1	Empty
2 to 7	Empty
8 to 14	Empty
15 to 20	• 4x HT U Deepwell Plate, Barcoded, 2.2mL
21 to 26	• 4x MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL • 4x HT 8-Strip Tubes, Clear, 0.2 mL
27 to 32	• HT Conductive 50 µL Filter Tips (4x96) • HT Conductive 300 µL Filter Tips (96)
33 to 38	HT Conductive 1 mL Filter Tips (96)
39 to 44	Empty
45 to 50	Empty
53	4x PCR MM (Positions 29 to 32)

References

1) Mackay, I.M. (2004). Real-time PCR in the microbiology laboratory. *Clin Microbiol Infect.* 10(3), 190–212.
2) Pyrc, K., Berkhout B. and van der Hoek L. (2006). The Novel Human Coronaviruses NL63 and HKU1. *J Virol*. 81(7), 3051-3057.
3) Trombetta, H., Faggion, H.Z., Leotte, J., Nogueira, M.B., Vidal, L.R.R. and Raboni, S.M. (2016). Human coronavirus and severe acute respiratory infection in Southern Brazil. *Pathog Glob Health* 110(3): 113-118.
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2021-11-05

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For Emergency Use Authorization Only

Rx Only

For Prescription Use Only.

IVD

For *in vitro* diagnostic (IVD) use

Consult instructions for use. Electronic Instruction for Use (IFU) is available on veladx.com at https://www.veladx.com/product/qpcr-respiratory-viruses/virokey-sars-cov-2-rt-pcr-test-v2.html

Please contact your local Vela Diagnostics representative at 1-877-593-7528 if you require a printed copy free of charge or need technical support to access the package insert.

Intended Use

ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal or nasopharyngeal aspirates, nasal washes and bronchoalveolar lavage samples collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The ViroKey. SARS-CoV-2 RT-PCR Test v2.0 is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The ViroKey. SARS-CoV-2 RT-PCR Test v2.0 is only for use under the Food and Drug Administration's Emergency Use Authorization.

n = 3 Pooling: The following indication is authorized under the FDA's Pooling and Serial Testing Amendment [Amendment Letter] for use in laboratories certified under CLIA to perform high complexity tests. The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 test is intended for the qualitative detection of RNA from the SARS-CoV-2 in pooled samples containing aliquots of transport media from up to 3 individual human anterior nasal swab specimens that were collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP from individuals without symptoms or other reasons to suspect COVID-19, when tested at least once per week as part of a serial testing program. This indication is authorized with the testing guidelines within this Instructions For Use. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be reported as presumptive positive or tested individually prior to reporting a result.

Principle

The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 uses TaqMan® probe chemistry for real-time RT-PCR detection of viral nucleic acid extracted from respiratory specimens using the ViroKey® SX Virus Total Nucleic Acid Kit (4x48) on an automated workflow using the liquid handler *Sentosa*® SX101 alone or *Sentosa*® SX101 and Thermo Fisher™ KingFisher™ Flex instrument combination; or the ViroKey® HT Virus Total Nucleic Acid Kit (4x96) on an automated workflow using the liquid handler Hamilton Microlab® STAR™ instrument. The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 contains two primer/probe sets that target the *ORF1a* (FAM reporter dye in the Green fluorescence channel) or *N* (HEX reporter dye in the Orange fluorescence channel) gene sequences of RNA from SARS-CoV-2 virus. The assays also contain primers and a probe to detect an extraction control (EC) sequence, which is a non-human synthetic DNA fragment added to each sample (reporter dye in the Red fluorescence channel).

Nucleic acids extracted from specimens are reverse transcribed into cDNA, and cDNA sequences that are complementary to the oligonucleotide primers are amplified by polymerase chain reaction using the supplied enzyme mixes (tubes RNA4 M2 and RNA4 M3; or tubes HT RNA M2 and HT RNA M3) on the *Sentosa*® SA201 Real-Time PCR system with the *Sentosa*® SA201 Reporter software. If the target nucleic acids are present and amplified, the probe(s) will anneal to specific complementary sequences located between the corresponding forward and reverse primers during the PCR process. During the extension phase of the PCR, the 5' nuclease activity of DNA polymerase degrades the probe bound to the specific target, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Probes specific to each target generate a fluorescent signal at different wavelengths, enabling the instrument to differentiate between the signals. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the *Sentosa*® SA201 Real-Time PCR system with the *Sentosa*® SA201 Reporter software or the Applied Biosystems 7500 Fast Dx Real-Time PCR System with ABI SDS Software Version 1.4.1.

Materials Provided and Storage

The following table lists the contents of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) (PN: 301068).

Item	Quantity	Volume / tube	Shipping Condition	Storage Condition
SARS-CoV-2 v2 M1	8	60 µL	Dry ice	-25°C – -15°C
RNA4 M2	8	700 µL	Dry ice	-25°C – -15°C
RNA4 M3	8	125 µL	Dry ice	-25°C – -15°C
NC5	8	600 µL	Dry ice	-25°C – -15°C
SARS-CoV-2 v2 PC	8	300 µL	Dry ice	-25°C – -15°C
EC8	8	600 µL	Dry ice	-25°C – -15°C

The following table lists the contents of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) (PN: 301085).

Item	Quantity	Volume / tube	Shipping Condition	Storage Condition
HT SARS-CoV-2 M1	4	115 µL	Dry ice	-25°C – -15°C
HT RNA M2	4	1400 µL	Dry ice	-25°C – -15°C
HT RNA M3	4	115 µL	Dry ice	-25°C – -15°C
HT NC	4	300 µL	Dry ice	-25°C – -15°C

HT SARS-CoV-2 PC	4	300 µL	Dry ice	-25°C – -15°C
HT EC	4	1200 µL	Dry ice	-25°C – -15°C

Warnings and Precautions

- This test is for use under an Emergency Use Authorization Only
- For Prescription Use Only.
- For *in vitro* diagnostic use only (IVD).
- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform high complexity tests
- The ViroKey® SARS-CoV-2 RT-PCR Test v2.0 has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- The performance of these tests was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- The product is to be used by qualified and trained laboratory personnel only.
- Strict compliance with the instructions for use is required for optimal PCR results.
- Each tube of reagent is designed for 50 reactions.
- Do not use expired kit components. Expiration dates are printed on the box and labels of all components. RNA4 M3 / HT RNA M3 are enzymes, which are in liquid state. Except RNA4 M3 and HT RNA M3, the rest of the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 components should be thawed completely at room temperature (approximately 15°C – 25°C) for up to 30 minutes before use.
- RNA4 M3 / HT RNA M3 should be used directly out of the freezer or kept on ice when performing reagent preparation. Handle carefully to avoid contamination and store the remaining RNA4 M3 / HT RNA M3 immediately after use at ≤ −20°C for subsequent reactions.
- All reagents, except RNA4 M2 / HT RNA M2 and RNA4 M3 / HT RNA M3, require thorough mixing by quick vortex. Mix RNA4 M2 / HT RNA M2 and RNA4 M3 / HT RNA M3 by gentle inversion. Centrifuge all tubes briefly to collect the contents at the bottom of the tubes. Avoid foaming of the reagents.
- All relevant documents (refer to “Resources” section) should be read thoroughly before performing the assay.
- Mutations that arise within the highly conserved regions of the viral genome covered by the kit’s primers and / or probes may result in failure to detect the presence of the virus.
- May cause allergic skin reactions.
- May be harmful if swallowed.
- Use personal protective equipment as required.
- For additional information, please refer to the Material Safety Data Sheet (MSDS).
- All samples and waste should be considered potentially infectious. Clean and disinfect all work surfaces thoroughly with disinfectants recommended by local authorities.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection when handling samples and kit reagents.
- Clean and decontaminate work area and instruments, including pipettes, with commercially available decontamination products.
- Avoid microbial and nuclease contamination of reagents when removing aliquots from reagent bottles. Use sterile disposable pipette tips.
- To avoid environmental contamination by amplicons, do not remove the PCR seal after amplification.
- Wash hands thoroughly after handling biological samples and kit reagents.

Workflow

For more information about n = 3 pooling strategy for implementation and monitoring see the IFU before proceeding with the PCR workflow.

Sentosa® SX101 workflow

The workflow starts with sample off-board lysis, followed by the extraction of nucleic acids and RT-PCR set up with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate using the Sentosa® SX101.
The ViroKey® SX Virus Total Nucleic Acid Kit (4x48) is used for nucleic acid extraction with the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) for RT-PCR setup.
After sample lysis and RT-PCR set-up, the MicroAmp® Fast Optical 96-Well Reaction is sealed and then transferred to the Sentosa® SA201 or Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument for PCR amplification followed by data analysis.
The Sentosa® SA201 is a rebranded version of the Applied Biosystems® 7500 Fast Dx Real-Time PCR System, thus the workflow is interchangeable between the two instruments.

Thermo Fisher™ KingFisher™ Flex workflow

The workflow starts with sample off-board lysis, followed by lysis incubation and ViroKey® SX Virus Total Nucleic Acid Kit (4x48) buffer preparation on the *Sentosa*® SX101, nucleic acid extraction on the Thermo Fisher™ KingFisher™ Flex instrument, and finally RT-PCR set up with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate using the *Sentosa*® SX101.

The ViroKey® SX Virus Total Nucleic Acid Kit (4x48) is used for nucleic acid extraction with the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (8x48) for RT-PCR setup.

After sample lysis and RT-PCR set-up, the MicroAmp® Fast Optical 96-Well Reaction and then transferred to the *Sentosa*® SA201 PCR amplification respectively. This is followed by data analysis using *Sentosa*® SA201 Reporter.

Hamilton Microlab® STAR™ workflow

The workflow starts with sample off-board lysis, followed by the extraction of nucleic acids and RT-PCR set up with the extracted nucleic acids in the MicroAmp® Fast Optical 96-Well Reaction Plate using the Hamilton Microlab® STAR™.
The ViroKey® HT Virus Total Nucleic Acid Kit (4x96) is used for nucleic acid extraction with the ViroKey® SARS-CoV-2 RT-PCR Test v2.0 (4x96) for RT-PCR setup.

After sample lysis and RT-PCR set-up, the MicroAmp® Fast Optical 96-Well Reaction is sealed and then transferred to the *Sentosa*® SA201 for PCR amplification followed by data analysis.

Regulatory status

This test is for use under an Emergency Use Authorization Only.
For Prescription Use Only.

Symbols

Symbol	Description
	Contains reagents sufficient for <*n*> tests

Symbol	Description
	Use-by date
IVD	For *in vitro* diagnostic (IVD) use
Rx Only	Prescription device
REF	Catalog number
COMP	Component
NUM	Number
CONT	Content
LOT	Lot number
CONTROL	Control
CONTROL -	Negative control
CONTROL +	Positive control
MAT	Document / label identification number
	Temperature limitations
	Legal manufacturer
	Refer to instructions for use

Resources

The latest edition of the MSDS and instructions for use of ViroKey® SARS-CoV-2 RT-PCR Test v2.0, ViroKey® SX Virus Total Nucleic Acid Kit (4x48) and ViroKey® HT Virus Total Nucleic Acid Kit (4x96) are available for download at www.veladx.com by logging in as an authorized user or requesting them via email.

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