

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



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REF

301068



8x48 tests

MAT

PS103999C



Consult instructions for use

Version 1.1

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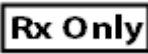












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Kit contents

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

Symbols

	Contains reagents sufficient for <N> tests
	Expiration date
	<i>In vitro</i> 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

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Storage

The components of the ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 should be stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and are stable until the expiration date stated on the label. RNA4 M3 is an enzyme, which is in liquid state. Except RNA4 M3, all reagents should be thawed completely before use.

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

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. 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.

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.
- 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 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.
- 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 is an enzyme, which is in liquid state. Except RNA4 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 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 immediately after use at $\leq -20^{\circ}\text{C}$ for subsequent reactions.
- All reagents, except RNA4 M2 and RNA4 M3, require thorough mixing by quick vortex. Mix RNA4 M2 and RNA4 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).

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- 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 and the ViroKey™ SARS-CoV-2 RT-PCR Test v2.0, 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 of a ready-to-use system for the detection of SARS-CoV-2 RNA extracted with the ViroKey™ SX Virus Total Nucleic Acid Kit on the *Sentosa*® SX101. 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 (NC) and a positive control (SARS-CoV-2 v2 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 on *Sentosa*® automated workflow using the liquid handler *Sentosa*® SX101 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) 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 humans¹. Other symptoms include rhinitis, cough, sore throat, and fever².

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 confirmed⁴. In February 2020, the International Committee on Taxonomy of Viruses named the novel coronavirus SARS-CoV-2⁵.

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 has not been evaluated for patients receiving intranasally administered influenza vaccine.

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- 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.
- 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 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/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: 

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- (A) Authorized laboratoriesⁱ 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 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.
- (F) 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.
- (G) 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.

ⁱ 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

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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

Negativity / positivity

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

Fluorescence channel	Expected C _T 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	< 22.0, > 30.0 or no Ct	22.0 – 30.0	< 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	< 22.0, > 30.0 or no Ct	22.0 – 30.0	< 10.0, > 38.0 or no Ct	10.0 – 38.0
Red (extraction control, EC)	< 23.0, > 32.0 or no C _t	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

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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

Performance characteristics

Analytical sensitivity

The analytical limit of detection (LoD) of ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 was assessed with the *Sentosa*® workflow, starting with sample extraction using the ViroKey™ SX Virus Total Nucleic Acid Kit 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 2.0×10^4 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 $\geq 95\%$ for 20 samples for one of the targets. (**Table 1**). The overall LoD of the ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 is 200 GE/mL.

Table 1. Results from confirmatory LoD study for the ViroKey™ SARS-CoV-2 RT-PCR Test v2.0.

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

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. 46,636 complete sequences (4,916 from GenBank and 41,720 from GISAID – sequences downloaded on June, 18 2020) were aligned against ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 primers and probes. The sequences were aligned with MAFFT (<https://mafft.cbrc.jp/alignment/server/>).

Out of the 4,916 complete SARS-CoV-2 sequences from NCBI database as of 18th June 2020,

- ORF1a primers and probes had 100% match to 4,900 out of 4,916 sequences (99.67%). For the remaining 16 sequences (0.33%), the primers and probes have at most 1 mismatch with these sequences.

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- N-gene primers and probes had 100% match to 4,901 out of 4,916 sequences (99.69%). For the remaining 15 sequences (0.31%), the primers and probes have at most 1 mismatch with these sequences.

Out of the 41,720 complete SARS-CoV-2 sequences from GISAID database as of 18th June 2020,

- ORF1a primers and probes had 100% match to 41,466 out of 41,720 sequences (99.39%). For the remaining 254 sequences (0.61%), the primers and probes have at most 1 mismatch with these sequences.
- N-gene primers and probes had 100% match to 41,472 out of 41,720 sequences (99.41%). For the remaining 248 sequences (0.59%), 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 analyzed SARS-CoV-2 sequences in the GenBank (n=4,916) and in GISAID (n=41,720) 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 2**. 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 2. *In silico* analysis for ViroKey™ SARS-CoV-2 RT-PCR Test v2.0.

Microorganism	Genbank Acc No.	<i>In silico</i> analysis for % identity/homology					
		N			ORF1a		
		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		

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Microorganism	Genbank Acc No.	In silico analysis for % identity/homology					
		N			ORF1a		
		Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
Human adenovirus B2	NC_011202.1	No alignment was found			No alignment was found		
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		
<i>Chlamydomonas reinhardtii</i>	NC_002180.1	No alignment was found			No alignment was found		
<i>Haemophilus influenzae</i>	NZ_LN831035.1	65%	NA	NA	NA	NA	60%
<i>Legionella pneumophila</i>	NZ_LR134380.1	65%	68%	NA	67%	NA	56%
<i>Mycobacterium tuberculosis</i>	NC_000962.3	No alignment was found			67%	NA	NA
<i>Streptococcus pneumoniae</i> *	NZ_LN831051.1	80%	68%	NA	NA	NA	52%
<i>Streptococcus pyogenes</i>	NC_002737.2	No alignment was found			NA	NA	60%
<i>Bordetella pertussis</i>	NC_018518.1	65%	NA	NA	62%	NA	NA

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Microorganism	Genbank Acc No.	In silico analysis for % identity/homology					
		N			ORF1a		
		Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
<i>Mycoplasma pneumoniae</i>	NZ_CP010546.1	No alignment was found			NA	NA	52%
<i>Pneumocystis jirovecii</i> (PJP)	GCF_001477535.1	No alignment was found			NA	NA	52%
<i>Candida albicans</i> *	GCF_000182965.3	65%	74%	67%	62%	NA	60%
<i>Pseudomonas aeruginosa</i>	GCF_000006765.1	65%	NA	NA	67%	NA	52%
<i>Staphylococcus epidermis</i>	GCF_000007645.1	NA	68%	NA	NA	71%	NA
<i>Staphylococcus salivarius</i>	CP013216.1	NA	74%	NA	No alignment was found		
<i>Leptospira borgpetersenii</i>	GCF_000013945	65%	NA	NA	NA	NA	56%
<i>Leptospira interrogans</i>	GCF_000092565	65%	79%	NA	No alignment was found		
<i>Leptospira santarosai</i> *	GCF_000313175	70%	89%	NA	NA	62%	NA
<i>Chlamydia psittaci</i>	NC_017287.1	No alignment was found			No alignment was found		
<i>Coxiella burnetii</i> (Q-Fever)	NC_002971.4	NA	68%	NA	62%	NA	60%
<i>Staphylococcus aureus</i>	NC_007795.1	70%	68%	NA	No alignment was found		
<i>Klebsiella pneumonia</i> *	GCF_000240185.1	80%	84%	NA	62%	NA	52%
<i>Corynebacterium diphtheriae</i>	NZ_LN831026.1	65%	68%	NA	NA	NA	56%
<i>Legionella longbeachae</i> *	GCF_000091785.1	65%	68%	NA	81%	67%	56%
<i>Bacillus anthracosis</i> (Anthrax)	GCF_000008445.1	NA	68%	NA	NA	NA	56%
<i>Moraxella catarrhalis</i>	NC_014147.1	85%	NA	NA	NA	62%	52%
<i>Neisseria elongata</i>	NZ_CP007726.1	70%	68%	NA	62%	NA	NA
<i>Neisseria meningitidis</i>	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-	NC_014470.1	No alignment was found			NA	57%	NA

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Microorganism	Genbank Acc No.	In silico analysis for % identity/homology					
		N			ORF1a		
		Forward Primer	Reverse Primer	Probe	Forward Primer	Reverse Primer	Probe
31/BGR/2008							
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%
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 3**.

Table 3. Potential cross-reactivity of the ViroKey™ SARS-CoV-2 RT-PCR Test v2.0.

Tested pathogens, strain (RNA)		Sample input	ORF1a	N	EC
Bacteria	<i>Haemophilus influenzae</i>	5x 10 ⁶ copies	0/3	0/3	3/3
	<i>Mycobacterium tuberculosis</i> , H37Ra	(1x 10 ⁶)	0/3	0/3	3/3
	<i>Streptococcus pneumoniae</i>		0/3	0/3	3/3

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Tested pathogens, strain (RNA)		Sample input	ORF1a	N	EC
	<i>Streptococcus pyogenes</i> Rosenbach	copies/ reaction)	0/3	0/3	3/3
	<i>Mycoplasma pneumoniae</i>		1/9*	1/9*	9/9
	<i>Legionella pneumophila</i>		0/3	0/3	3/3
	<i>Chlamydophila pneumoniae</i> strain CM-1		0/3	0/3	3/3
	<i>Bordetella pertussis</i>		0/3	0/3	3/3
Virus	Coronavirus (human), 229E		0/3	0/3	3/3
	Coronavirus (human), 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
	Middle East Respiratory Syndrome (MERS)		0/3	0/3	3/3
	Human metapneumovirus (hMPV)		0/3	0/3	3/3
	Human adenovirus 1, Adenoid 71		1/9*	0/9	9/9
	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 (H1N1), A/Swine/Iowa/15/30		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

* Initial testing with 3 replicates showed 1/3 positive for one or both targets for *Mycoplasma pneumoniae* and 1/3 positive for ORF1a for human adenovirus 71. Additional 6 replicates were tested.

Cross-reactivity was observed with *Mycoplasma pneumoniae* and adenovirus 71

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 SARS-CoV-2 genomic RNA (from American Type Culture Collection (ATCC® part number VR-1986D) 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 4** for each interference substance. This study demonstrates that the potential interfering substances tested at specific concentrations as stated in **Table 4** have no impact on the performance of ViroKey™ SARS-CoV-2 RT-PCR Test v2.0.

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Table 4. List of potential interfering substances tested with ViroKey™ SARS-CoV-2 RT-PCR Test v2.0.

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	N.A.	2% (v/v)	100% (3/3)	100% (3/3)
Mucin		60 µg/mL	100% (3/3)	100% (3/3)
Pooled human nasal wash		N.A.	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 and *Sentosa*® SA201 Real-Time PCR Instrument. The results are summarized in **Table 5**.

Table 5: 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	1.8x10 ⁵ NDU/mL	N/A

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MERS-CoV	Swab	N/A	ND
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NDU/mL: RNA NAAT detectable units/mL

N/A: Not Applicable

ND: Not Detected

Clinical performance

The clinical validation study of ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 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 on the Sentosa SX101 instrument and detected with ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 on the Sentosa® SA201.

A total of 68 nasopharyngeal samples were tested and the results are summarized in **Table 6**. The performance of the ViroKey™ SARS-CoV-2 RT-PCR Test v2.0 was calculated based on the result interpretation table on page 10, 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 6. Summary of clinical performance results

		Comparator Results	
		Positive	Negative
ViroKey™ SARS-CoV-2 RT-PCR Test v2.0	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%

Workflow

The workflow starts with 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.

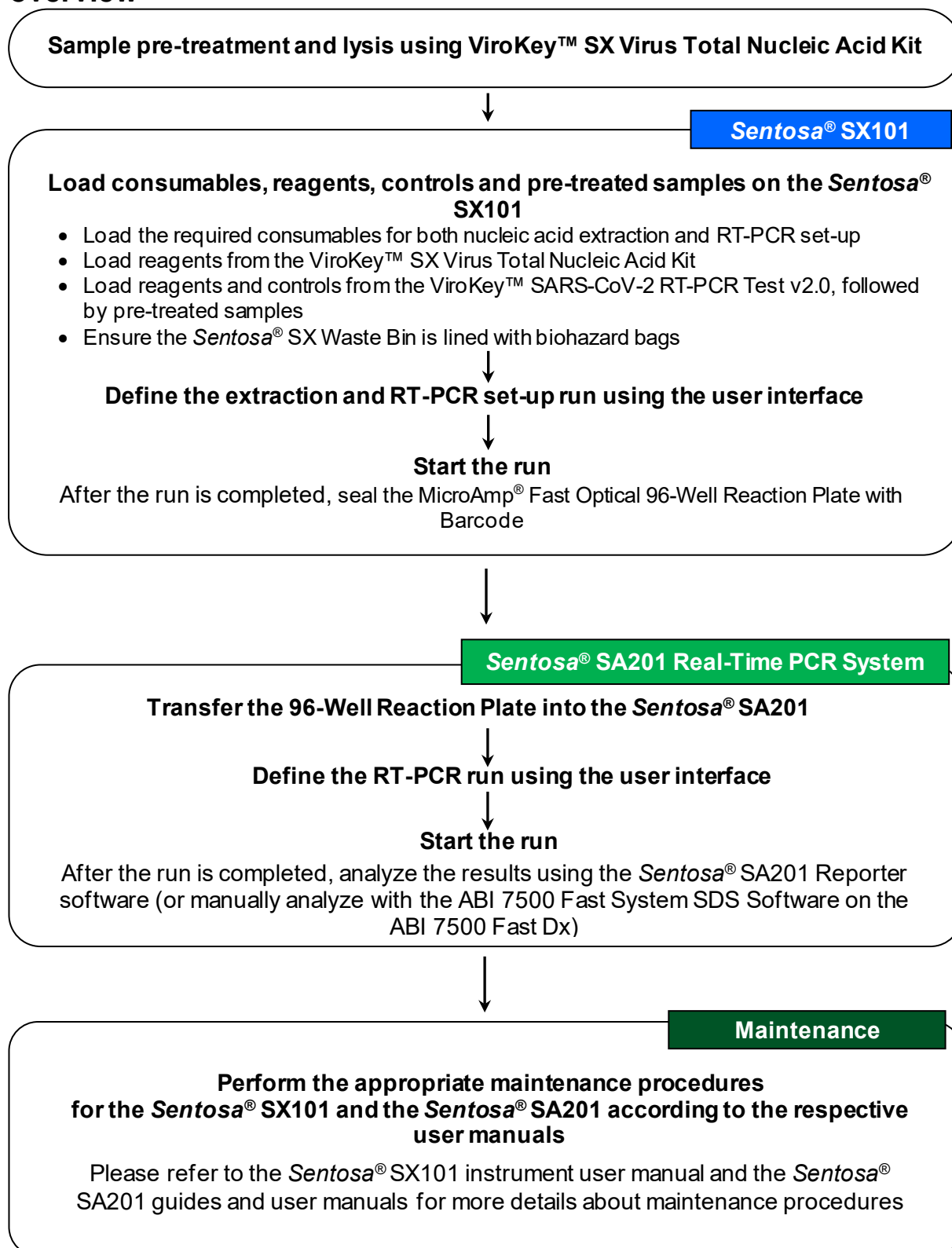
The ViroKey™ SX Virus Total Nucleic Acid Kit is used for nucleic acid extraction.

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 this instructions for use.

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

Flowchart: Workflow with *Sentosa*® SA201 Real-Time PCR System overview



Items to be supplied by user

Table 7. List of items to be supplied by user

Equipment / software	Description / use	Vela item no.
<i>Sentosa</i> [®] SX101 instrument ⁱ	Automated sample processing system	400089
Pipettes (adjustable) ⁱⁱ	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 ⁱⁱ	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
<i>Sentosa</i> [®] SA201 Real-time PCR instrument	Real-time and end-point thermal cycler for PCR, detection and analysis	400125
<i>Sentosa</i> [®] SA201 Series Software	To operate <i>Sentosa</i> [®] SA201 Real-time PCR Instrument and to perform PCR data analysis	460012
<i>Sentosa</i> [®] SA201 Reporter	To automate data analysis and result interpretation	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 ⁱⁱⁱ	4x48 tests	300677
<i>Sentosa</i> [®] SX Magnetic Separator	1 piece	400024
<i>Sentosa</i> [®] 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>Sentosa</i> [®] 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
<i>Sentosa</i> [®] SX 100mL Reservoir (50)	Set of 50 reservoirs	400027
<i>Sentosa</i> [®] SX 30mL Reservoir (50)	Set of 50 reservoirs	400028
<i>Sentosa</i> [®] SX Deepwell Plate 96/2000µL (20)	Set of 20 plates	400068
<i>Sentosa</i> [®] SX Biohazard Bag (100)	100 pieces	400033
Sterile pipette tips with filters	For pipetting buffers, reagents and/or samples	N/A

ⁱⁱ Ensure that the instruments have been checked and calibrated according to the manufacturer's recommendations.

ⁱⁱⁱ The ViroKey[™] SX Virus Total Nucleic Acid Kit is used for virus total nucleic acid extraction for use with the ViroKey[™] SARS-CoV-2 RT-PCR Test v2.0.

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Safe-Lock Tubes (1000)	For sample extraction. Alternatively, Sarstedt 1.5mL screwcap tubes (or tubes of equivalent dimensions) may be used	400031
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL ^{iv}	N/A	N/A
MicroAmp® Optical Adhesive Film ^v	N/A	N/A
MicroAmp™ Adhesive Film Applicator ^{vi}	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	N/A

^{iv} 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.

^v MicroAmp® Optical Adhesive Film from Applied Biosystems® (Cat. No. 4311971) **MUST** be used. Use only unexpired films.

^{vi} MicroAmp™ Adhesive Film Applicator from Thermo Fisher Scientific (Cat. No. 4333183) is recommended.

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/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

The ViroKey™ SX Virus Total Nucleic Acid Kit 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.

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 24.
- Ensure that all reagents of the ViroKey™ SX Virus Total Nucleic Acid Kit are not precipitated before use.
- Each tube of reagent in the ViroKey™ SARS-CoV-2 RT-PCR Test v.2.0 is designed for 48 reactions.

ViroKey™ SX Virus Total Nucleic Acid Kit

- 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 is an enzyme, which is in liquid state. Except RNA4 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).
- For software, use current version or higher

<i>Sentosa</i> ® SX101	Release 3.0 (Version 41.0.1.5)
<i>Sentosa</i> ® SA201 Series Software	Version 1.0.1 (for use with <i>Sentosa</i> ® SA201)
<i>Sentosa</i> ® SA201 Reporter	Version 1.3 (for use with <i>Sentosa</i> ® SA201)
ABI SDS Software	Version 1.4.1 (for manual data analysis with ABI 7500 Fast Dx)

Sample preparation

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 sample (NC) can be performed in one run of the ViroKey SARS-CoV-2 RT-PCR Test v2.0.

Samples

- Vortex swab in transport media for 30 seconds
- Transfer 230 μ L of samples to 1.5 mL Safe-Lock Tubes

Procedure

1. *Sample pre-treatment and lysis*

- 1.1. Prepare the reagents from the ViroKey™ SX Virus Total Nucleic Acid Kit 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.

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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 28.

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.

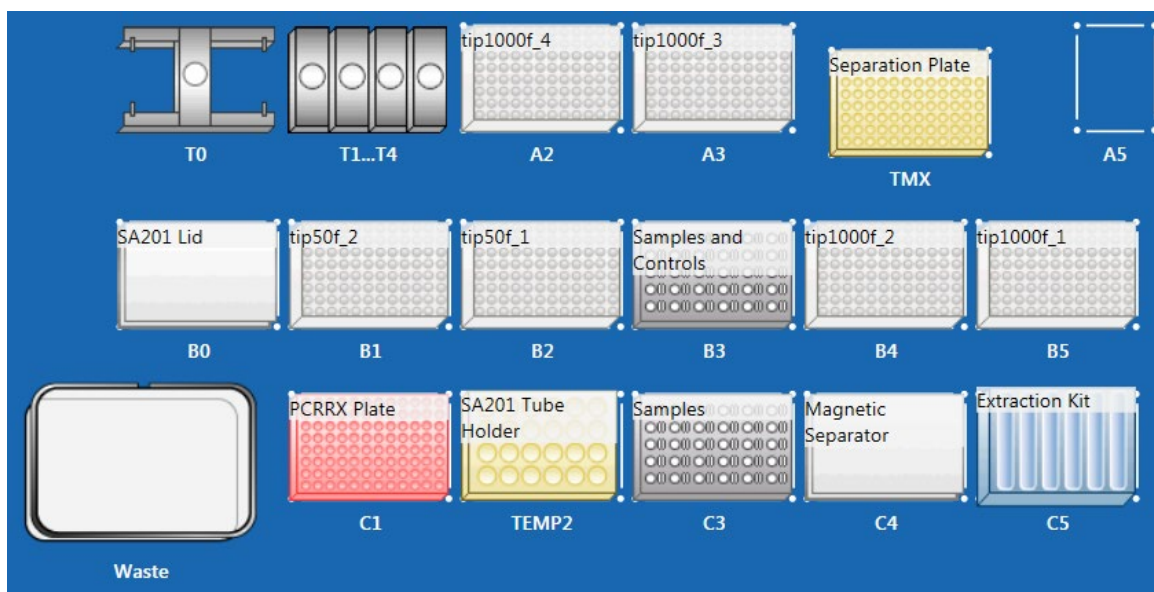



Figure 1. Layout of the *Sentosa*® SX101 platform for 96 tests

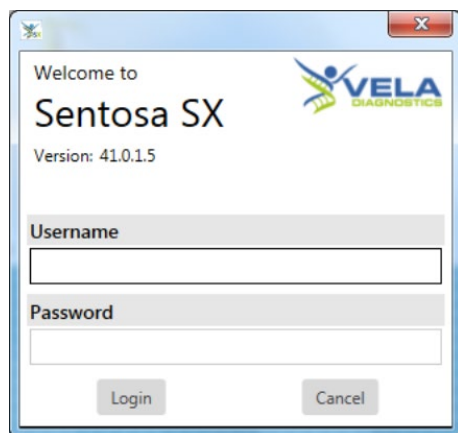
NOTE:

- 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.

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- 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.

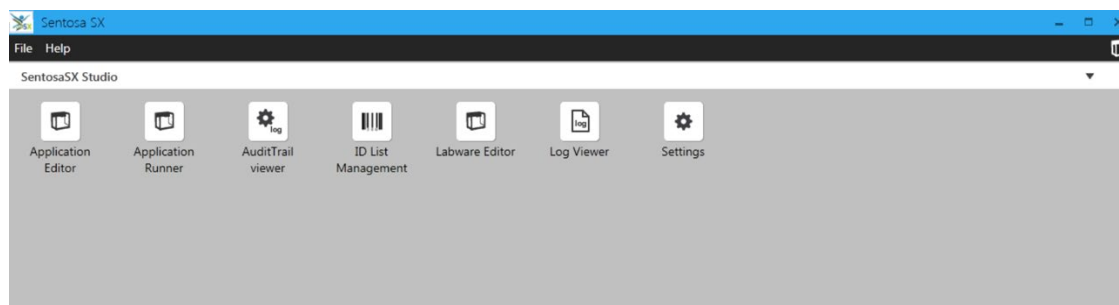
The “Login” window opens.



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	ViroKey™ SARS-CoV-2 RT-PCR Test v.20
48-1 ViroKey SARS-CoV-2-v2 v3-3	Up to 46 tests	1 set of reagents	1 set of reagents

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- 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**, TEMP2) according to the table below.

Application (v3-3 or higher)	<i>Sentosa</i> ® SX Reservoir Rack 7 (TEMP2)			
	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 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)	<i>Sentosa</i> ® SX Reservoir Rack 7 (TEMP2)					
	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.

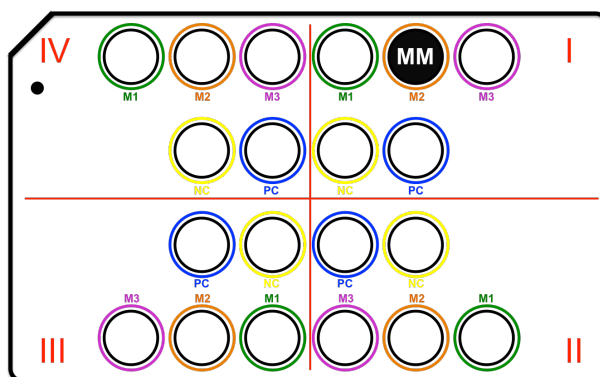
IMPORTANT: Correct placement of assay components is critical for successful completion of the test. Please follow on-screen prompt carefully for correct placement of components.

- 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.

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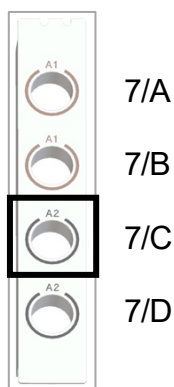


NOTE:

- 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 on the *Sentosa*® SX RR Module A1/A2 according to the table below.

Application (v3-3 or higher)	<i>Sentosa</i> ® SX Reservoir Rack 7 (TEMP2)			
	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 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.

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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**).

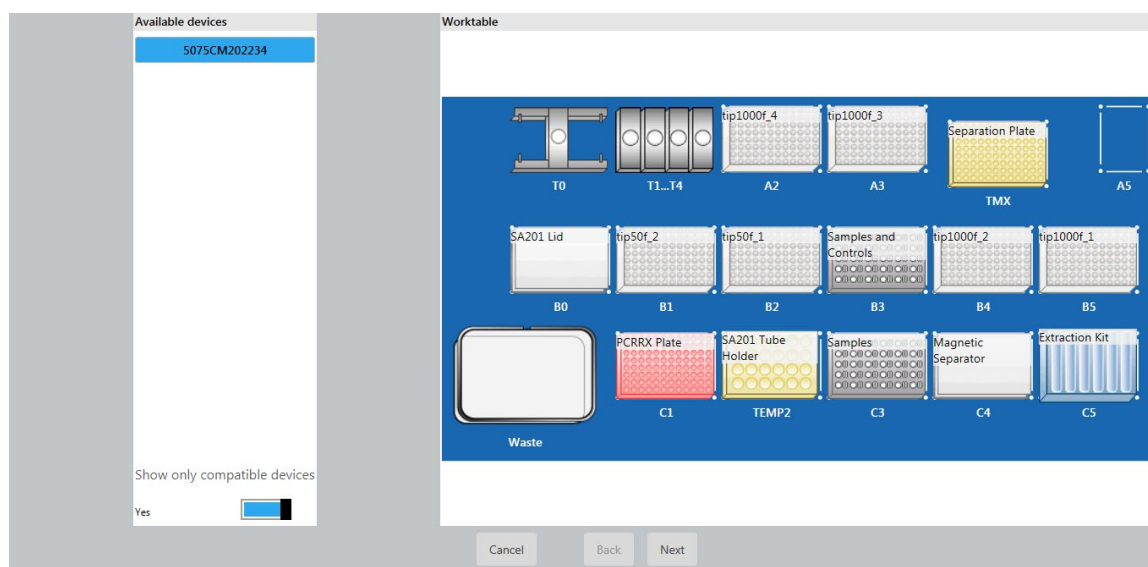


MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL



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”.



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- 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”.

Barcode reader settings
Use Barcodes
Yes ☒

Volume settings
Detect volumes ☒
Use required minimum volumes ☒
Input volumes manually ☒

Worktable settings
Detect tips
On ☒
Check labware placement
Yes ☒
Check vessel cap removed
Yes ☒

Worktable
T0 T1...T4 A2 A3 TMX A5
SA201 Lid tip50f_2 tip50f_1 Samples and Controls tip1000f_2 tip1000f_1
B0 B1 B2 B3 B4 B5
Waste PCRRX Plate SA201 Tube Holder Samples Magnetic Separator Extraction Kit
C1 TEMP2 C3 C4 C5

Cancel Select device Next

- 2.14. Enter the run name manually by checking the “Enter manually” check box. Click “OK” and then “Next”.

Labware info
Location: B3
Stack index: 1
Labware: dws/trth/EP_Rack_SafeLock_1_5
Name: Samples and Controls

Barcode Settings
Manual scan ☒

Vessels
A1 A2 A3 A4 A5 A6
B1 B2 B3 B4 B5 B6
C1 C2 C3 C4 C5 C6
D1 D2 D3 D4 D5 D6
71 %

ID list name(s)
Input list: 48 TG SARS-CoV-2 v3-4-1_Samples and Controls_1

Position	Name	ID 1	ID 2	Usage
1	NC			Input
2	PC			Input
3	Sample 1			Input
4	Sample 2			Input
5	Sample 3			Input
6	Sample 4			Input
7	Sample 5			Input
8	Sample 6			Input
9	Sample 7			Input
10	Sample 8			Input
11	Sample 9			Input
12	Sample 10			Input
13	Sample 11			Input
14	Sample 12			Input
15	Sample 13			Input
16	Sample 14			Input
17	Sample 15			Input
18	Sample 16			Input

Select ID list

Cancel Back Next

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- 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.

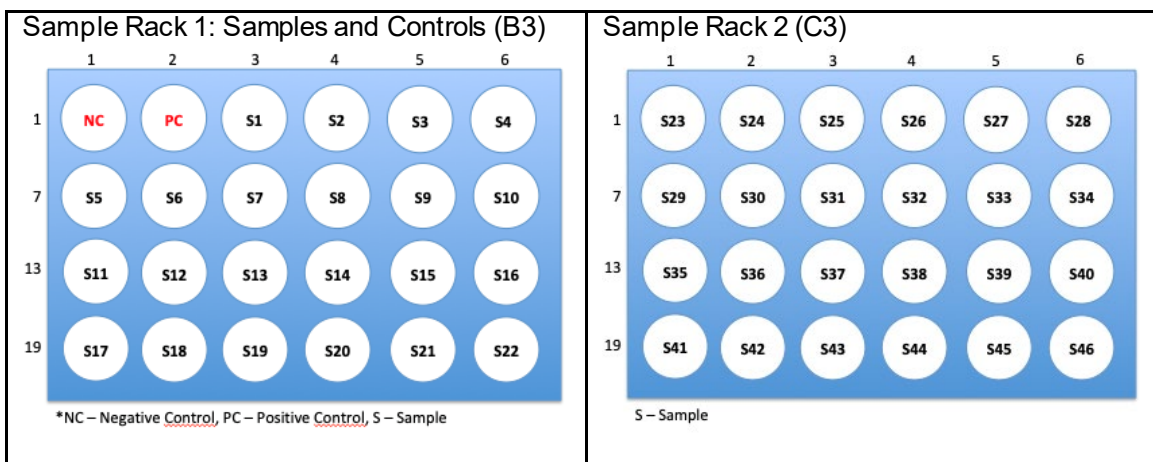
The screenshot shows the ViroKey software interface. On the left, there's a 'Labware info' section with fields for Location (B3), Stack index (1), Labware (divs/trth/EP_Rack_SafeLock_1_5), and Name (Samples and Controls). Below this is 'Barcode Settings' with a 'Manual scan' checkbox. The 'Vessels' section shows a 4x6 grid of green circles representing wells, with a zoom level of 71%. The main part of the interface is a table titled 'Position IDs' with columns: Position, Name, ID 1, ID 2, and Usage. The table lists 18 positions, from 1 to 18. Positions 1 and 2 are labeled 'NC' and 'PC' respectively. Positions 3 through 18 are labeled 'Sample 1' through 'Sample 16'. The 'ID 1' column contains barcode strings for NC and PC, and 'Sample' for the samples. The 'Usage' column is set to 'Input' for all positions. On the right, there's a 'Worktable' section showing a visual representation of the rack layout. At the bottom, there are 'Cancel', 'Back', and 'Next' buttons.

Position	Name	ID 1	ID 2	Usage
1	NC	V*202205*NC**0000000000		Input
2	PC	V*202224*PC**0000000000		Input
3	Sample 1	Sample		Input
4	Sample 2	Sample		Input
5	Sample 3	Sample		Input
6	Sample 4	Sample		Input
7	Sample 5	Sample		Input
8	Sample 6	Sample		Input
9	Sample 7	Sample		Input
10	Sample 8	Sample		Input
11	Sample 9	Sample		Input
12	Sample 10	Sample		Input
13	Sample 11	Sample		Input
14	Sample 12	Sample		Input
15	Sample 13	Sample		Input
16	Sample 14	Sample		Input
17	Sample 15	Sample		Input
18	Sample 16	Sample		Input

Application (v3-3 or higher)	Sentosa® SX Rack 0.5 + Adaptor / 1.5 / 2.0 mL		
	Sample rack (C3)		
	Position 1	Positions 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

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- 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*”.

Labware info
Location: C1
Stack index: 1
Labware: dws/thermo/EP_TT_Thermo_150_SC2
Name: PCRRX Plate

Barcode Settings
Manual scan

Vessels

ID list name(s)
Result list: 48-1 ViroKey SARS-CoV-2-v2 v3-3_PCRRX Plate_1
List ID: Sample

Position	Name	ID 1	ID 2	Usage
4	Sample-A4			Result
5	Sample-A5			Result
6	Sample-A6			Result
7	Sample-A7			Result
8	Sample-A8			Result
9	Sample-A9			Result
16	Sample-B4			Result
17	Sample-B5			Result
18	Sample-B6			Result
19	Sample-B7			Result
20	Sample-B8			Result
21	Sample-B9			Result
28	Sample-C4			Result
29	Sample-C5			Result
30	Sample-C6			Result
31	Sample-C7			Result

Cancel Back 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*”.

Labware info
Location: TEMP2
Stack index: 1
Labware: dws/trth/SA201 96Triplex
Name: SA201 Tube Holder

Barcode Settings
Manual scan

Vessels

ID list name(s)
Input list: 48-1 ViroKey SARS-CoV-2-v2 v3-3_SA201 Tube Holder_I_3
Position IDs

Position	Name	ID 1	ID 2	Usage
3	M1/M2/M3	V*202222*M1*15*0000000000		Input

Custom IDs

Name	Barcode
1	V*301070*PCRRX*0000000000*2020-12-12

Cancel Back Next

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- 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.

Labware info

Location: C3

Stack index: 1

Labware: divs/trth/EP_Rack_SafeLock_1_5

Name: Samples

Barcode Settings

Manual scan

Vessels

ID list name(s)

Input list: 48-1 ViroKey SARS-CoV-2-v2 v3-3_Samples_3

Position	Name	ID 1	ID 2	Usage
1	Sample 23	Sample		Input
2	Sample 24	Sample		Input
3	Sample 25	Sample		Input
4	Sample 26	Sample		Input
5	Sample 27	Sample		Input
6	Sample 28	Sample		Input
7	Sample 29	Sample		Input
8	Sample 30	Sample		Input
9	Sample 31	Sample		Input
10	Sample 32	Sample		Input
11	Sample 33	Sample		Input
12	Sample 34	Sample		Input
13	Sample 35	Sample		Input
14	Sample 36	Sample		Input
15	Sample 37	Sample		Input
16	Sample 38	Sample		Input
17	Sample 39	Sample		Input
18	Sample 40	Sample		Input

Select ID list

Worktable

Cancel Back Next

- 2.20. Scan the 2-D barcode on the ViroKey™ SX Virus Total Nucleic Acid Kit box that will be used in the run. Press / Click “*Next*”.

Labware info

Location: C5

Stack index: 1

Labware: divs/th/48 Samples MN Virus

Name: Extraction Kit

Barcode Settings

Manual scan

Vessels

ID list name(s)

Input list: 48-1 ViroKey SARS-CoV-2-v2 v3-3_Extraction Kit_3

Position	Name	ID 1	ID 2	Usage
6	D4	V=300679-EXTRX=0000000000=2020-		Input

Select ID list

Worktable

Cancel Back Next

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

2.18.1. *Sentosa*® SX Deepwell Plate 96/2000 µL (Location: TMX)

2.18.2. *Sentosa*® SX Rack 0.5 + Adaptor / 1.5 / 2.0 mL (Location: B3 and C3)

2.18.3. SA201 Tube Holder Rack (Location: TEMP C2)

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2.18.4. *Sentosa*® SX Reservoir Rack 7 (Location: C5)

At the last window, press / click “Run”.

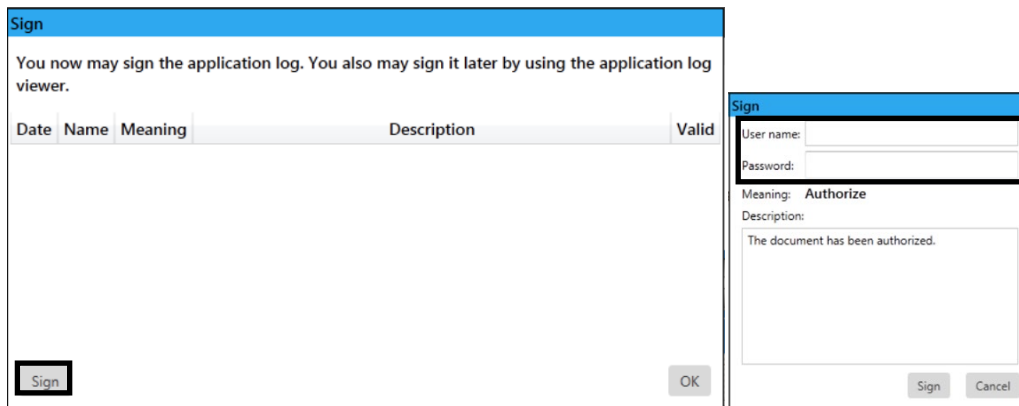
In the “*Please enter Reason*” pop-up window, enter a reason for the run in the textbox if applicable, if not, enter any alphanumeric character / symbol key and press / click “Save” to continue.



- 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 39 to 51) or Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with the ABI SDS Software for manual data analysis (pages 52 to 65).

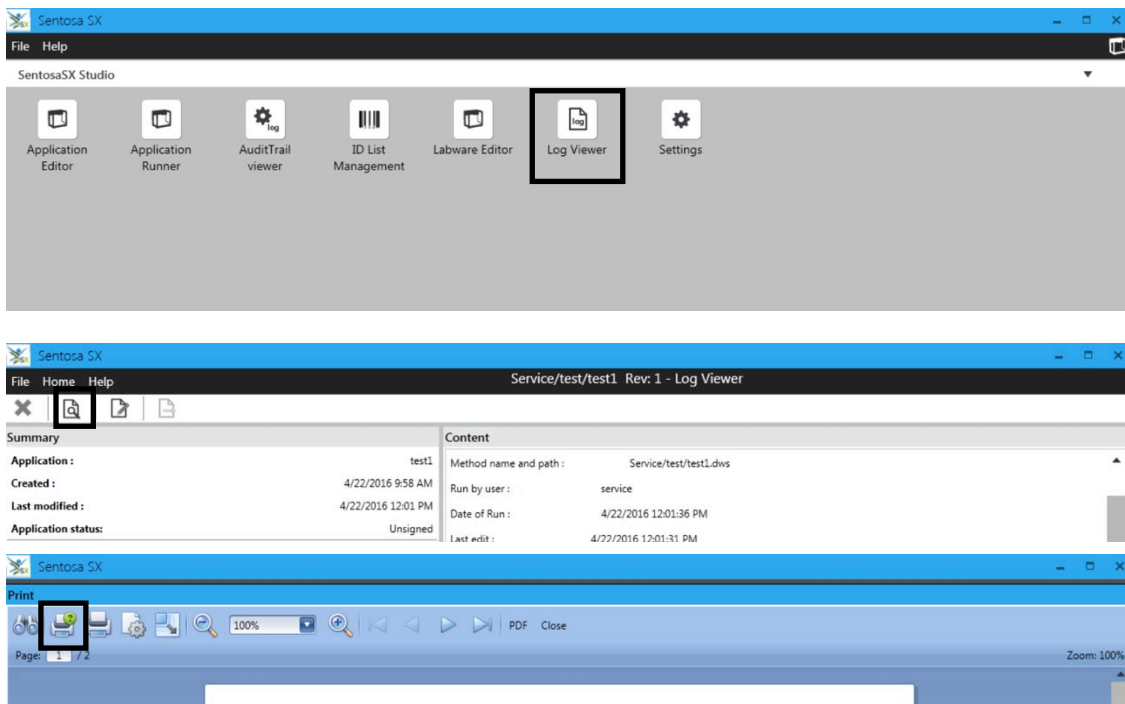
After RT-PCR and data analysis are complete, proceed to page 10 for result interpretation after data analysis.

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

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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.

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

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

- 3.1. Switch on the Sentosa® SA201 Real-Time PCR Instrument by pressing the power button on the instrument.

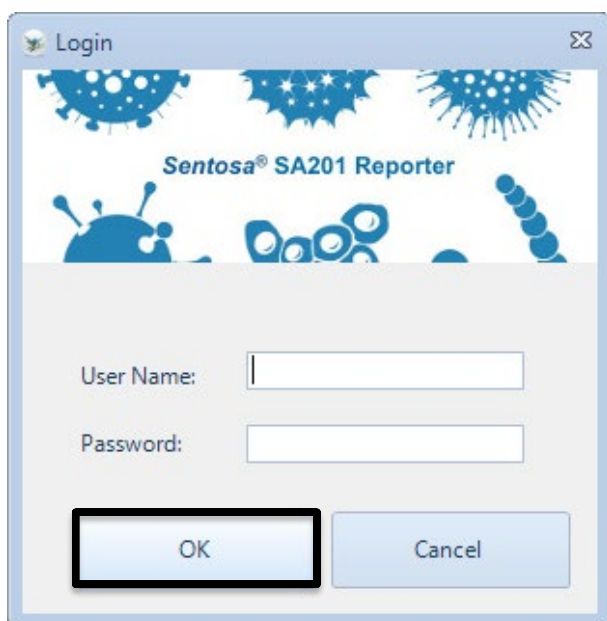
NOTE: Ensure the green indicator is lit and not flashing.



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



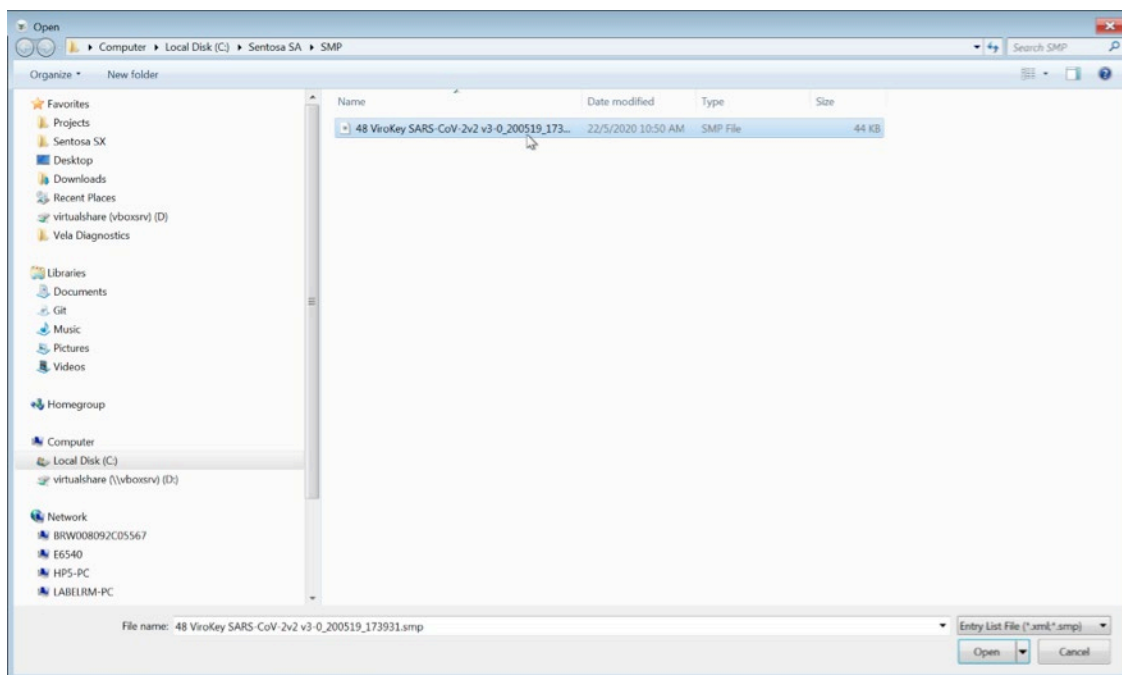
Type the user name and password, and then click “OK”.



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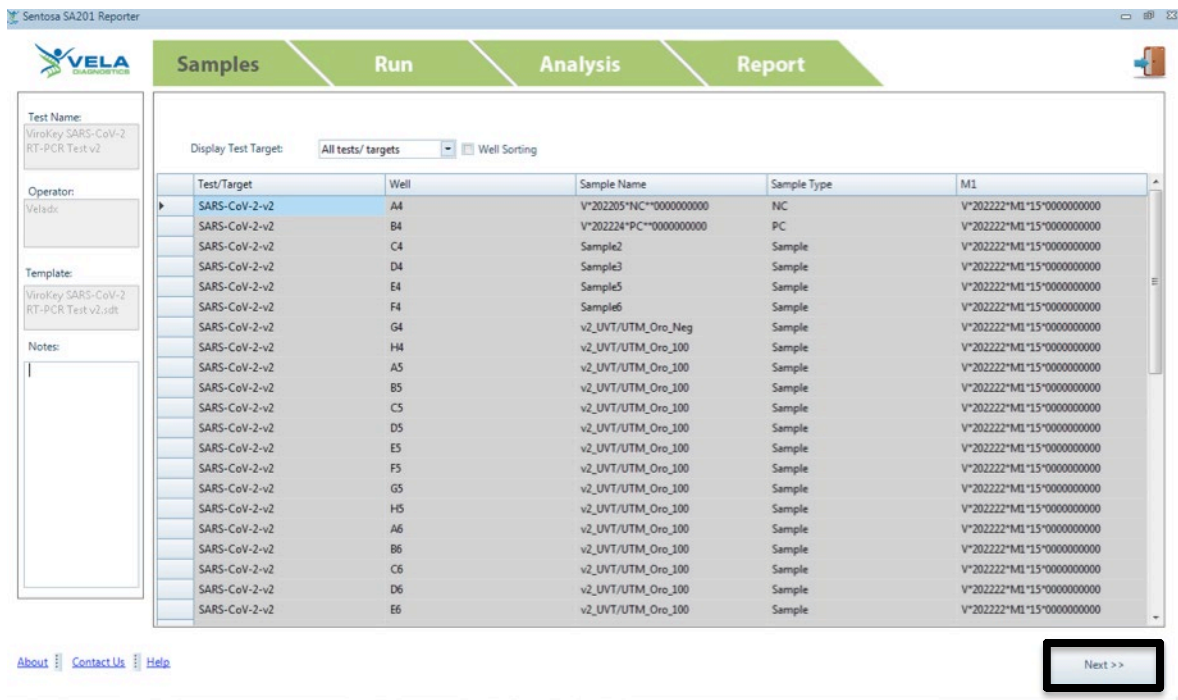
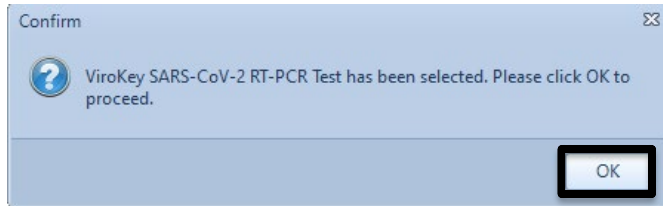
3.3. After logging in, “Access Mode” pop-up dialog is displayed.

Click “Import ID List” under “Access Mode” pop-up dialog to import the required “.smp” file generated by the *Sentosa*® SX101 instrument.



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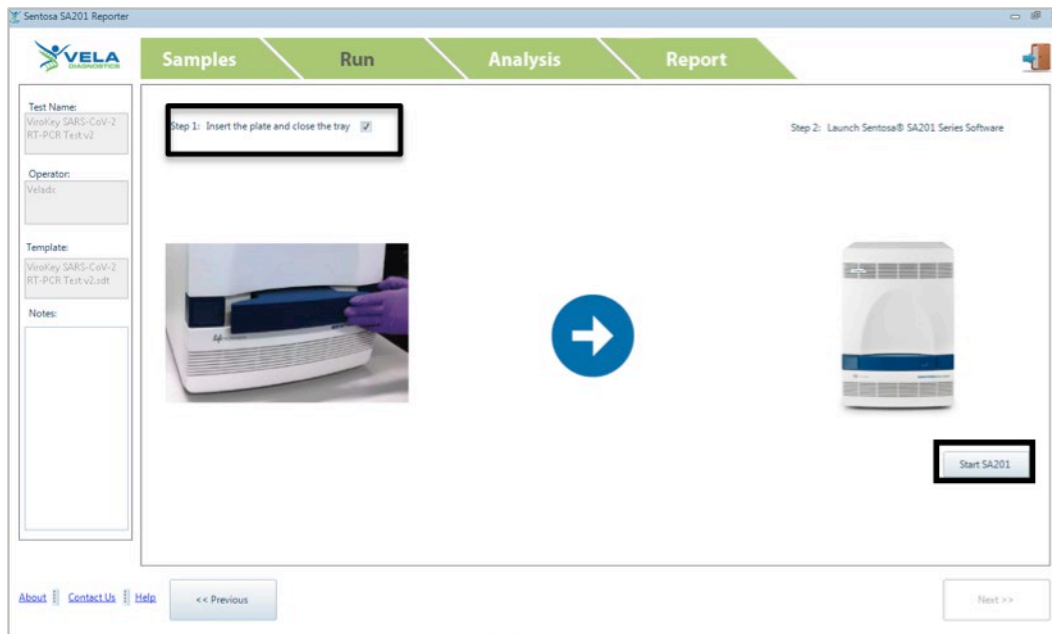
- 3.4. After the “.smp” file has been imported, the software will require a confirmation on the selected assay. Click “OK” to confirm the sample layout and the information displayed from the “.smp” file.



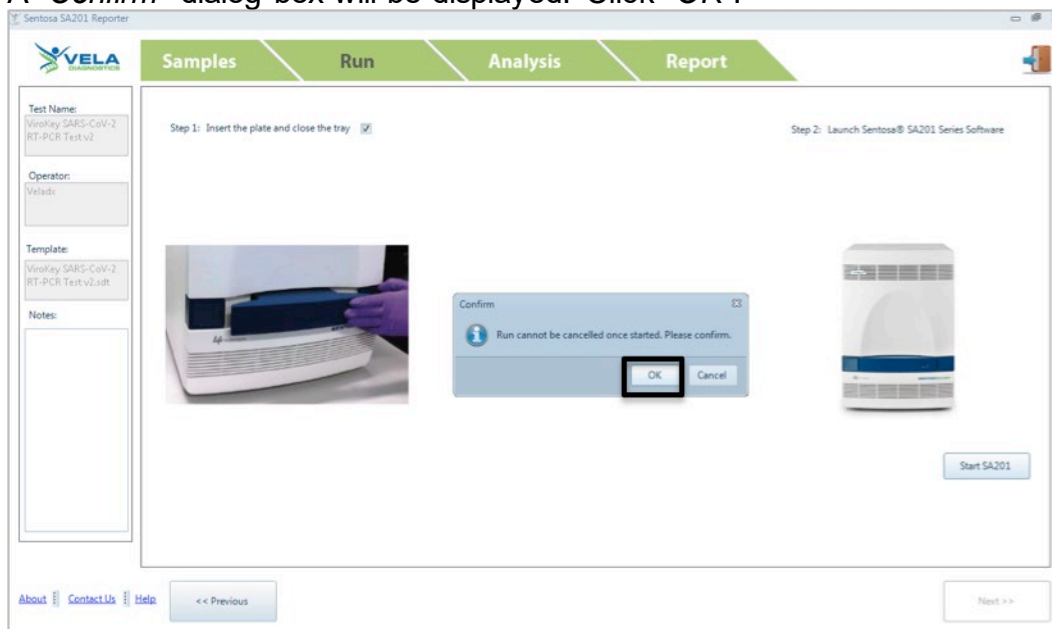
Click “Next”.

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- 3.5. Activate “Insert the plate and close the tray” checkbox to activate “Step 2: Launch Sentosa® SA201 Series Software”. Click on the “Sentosa® SA201 Real-Time PCR Instrument” icon.

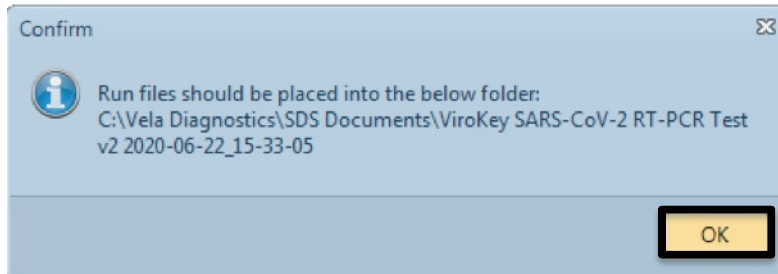


- 3.6. A “Confirm” dialog box will be displayed. Click “OK”.

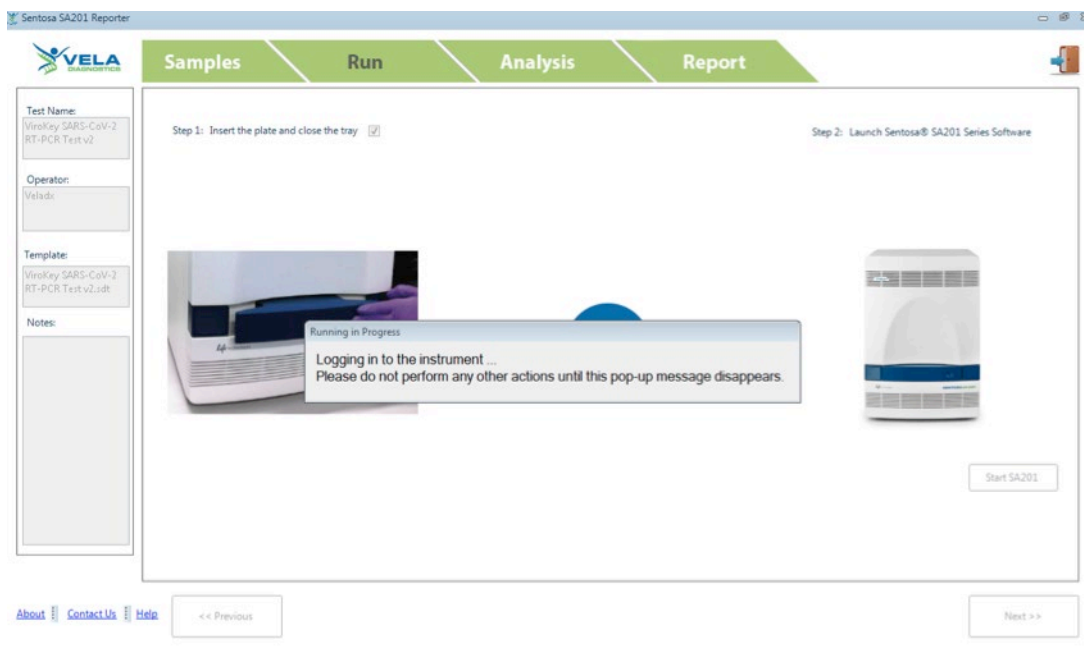


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3.7. Another “Confirm” dialog box will be displayed. Click “OK”.



3.8. 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.



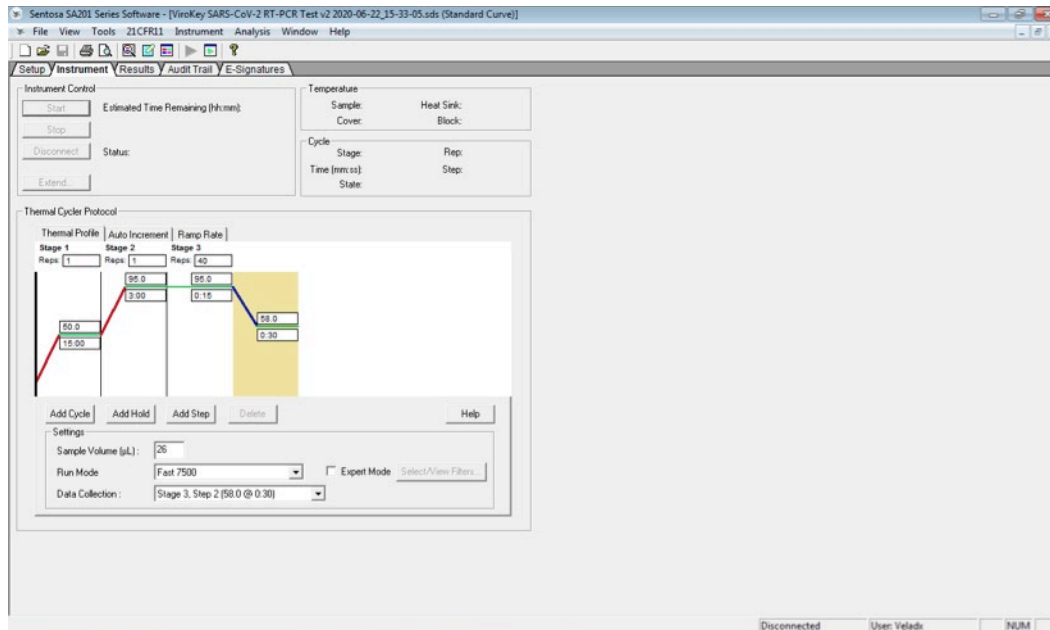
The following is the list of automated steps that will be performed on the *Sentosa*® SA201 Series Software:

- 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.

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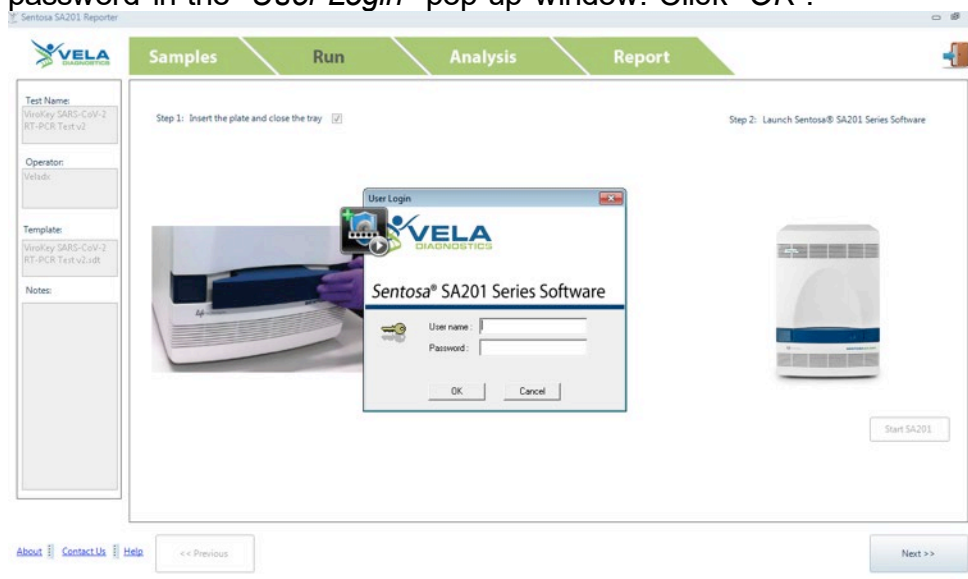
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Please do not perform any other action during the automated process. The run will be started on the *Sentosa*® SA201 instrument at the end of the process. This can be verified by selecting the “*Instrument*” tab and checking the “*Instrument Control*”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



3.9. [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”.

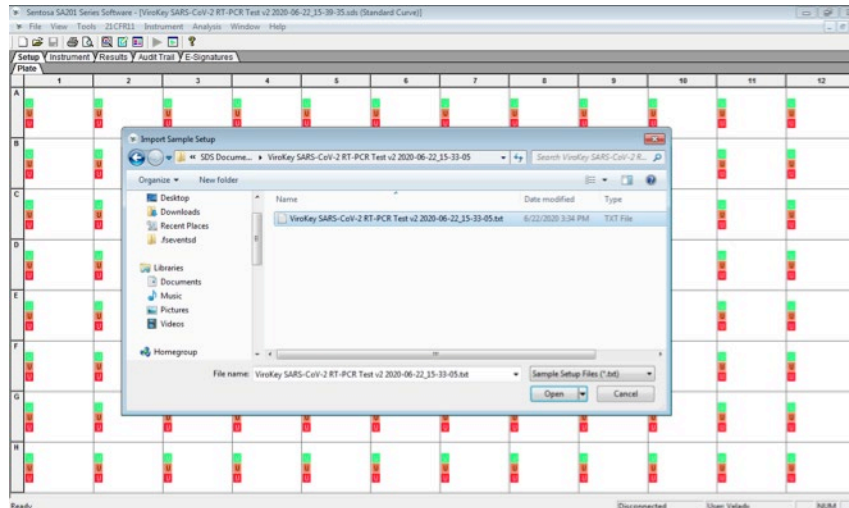


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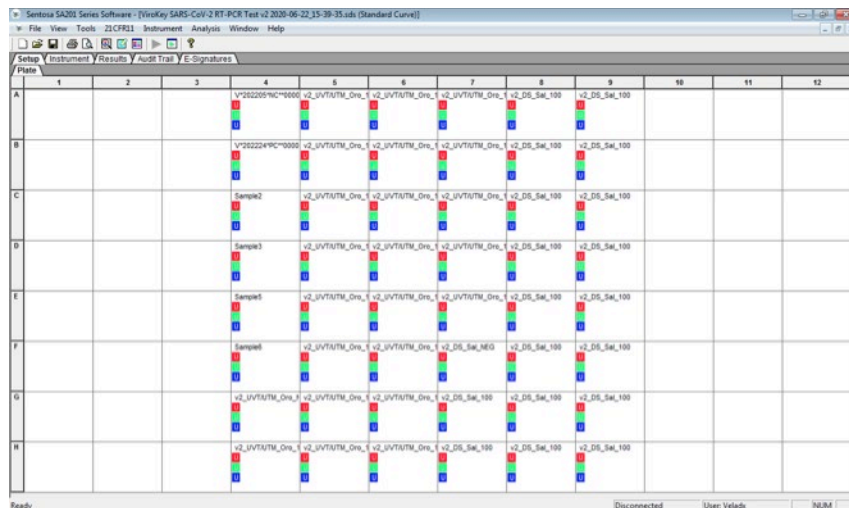
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- II. Select “File” from the main menu and “Import Sample Setup” from the drop-down list. “Import Sample Setup” pop-up window will be displayed. The sample file is given a default file name (“Sentosa SA201 Assay name PCR/RT-PCR Test YYYY-MM-DD_HH-MM-SS.txt”). Select the “.txt” file and click “Open”.

NOTE: DO NOT modify the generated “.txt” file as this will result in an error.

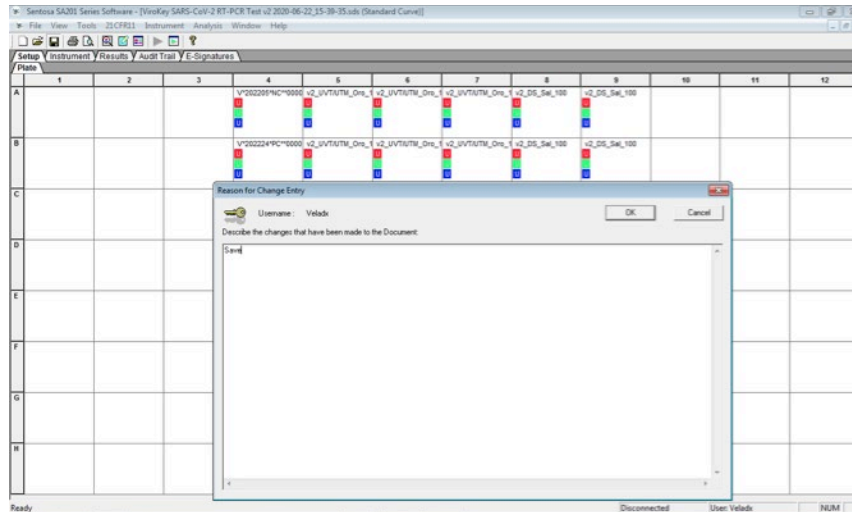


The sample layout will change according to the imported sample setup file.

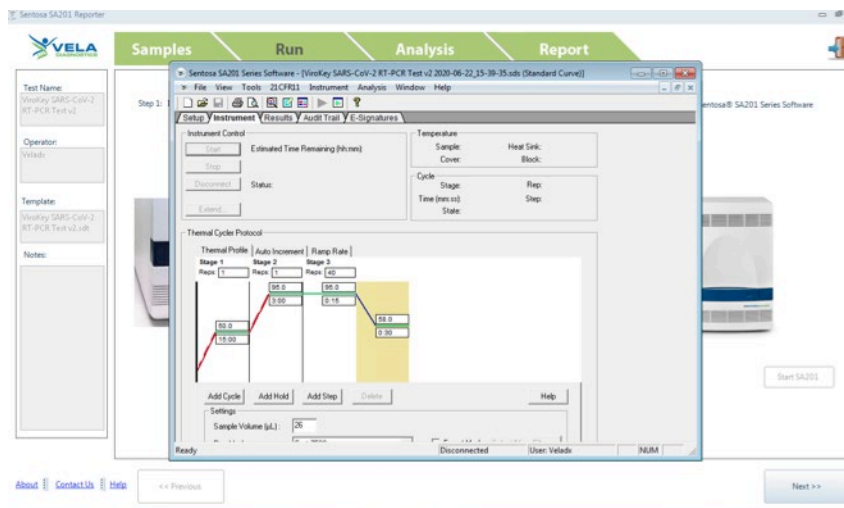


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- III. Select “File” from the main menu and “Save” from the drop-down list. “Reason for Change Entry” window will be displayed. Describe the changes made in the document or simply enter “Save file” or any other suitable text in the textbox and click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

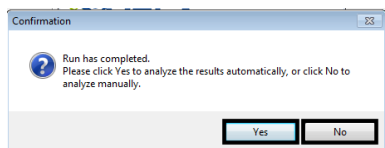


- IV. Select the “Instrument” tab and click “Start” to start the run.




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- 3.10. 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.

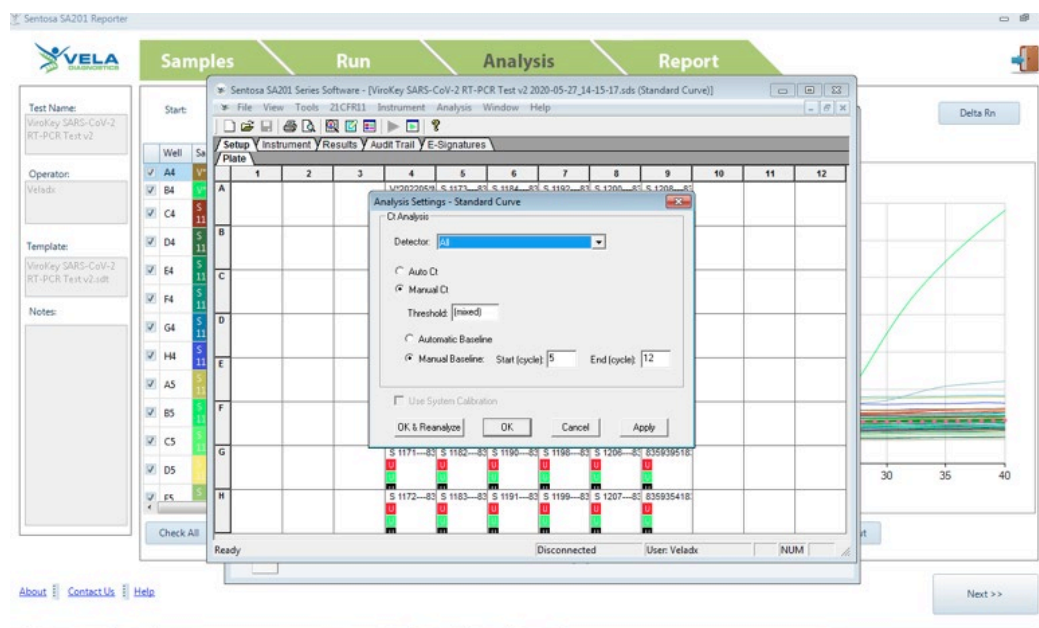


Click “Yes” to perform the result analysis automatically by using preconfigured analysis settings. A pop-up window titled “*Analysis in Progress*” will be displayed briefly, reminding the user not to perform any other actions.

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.



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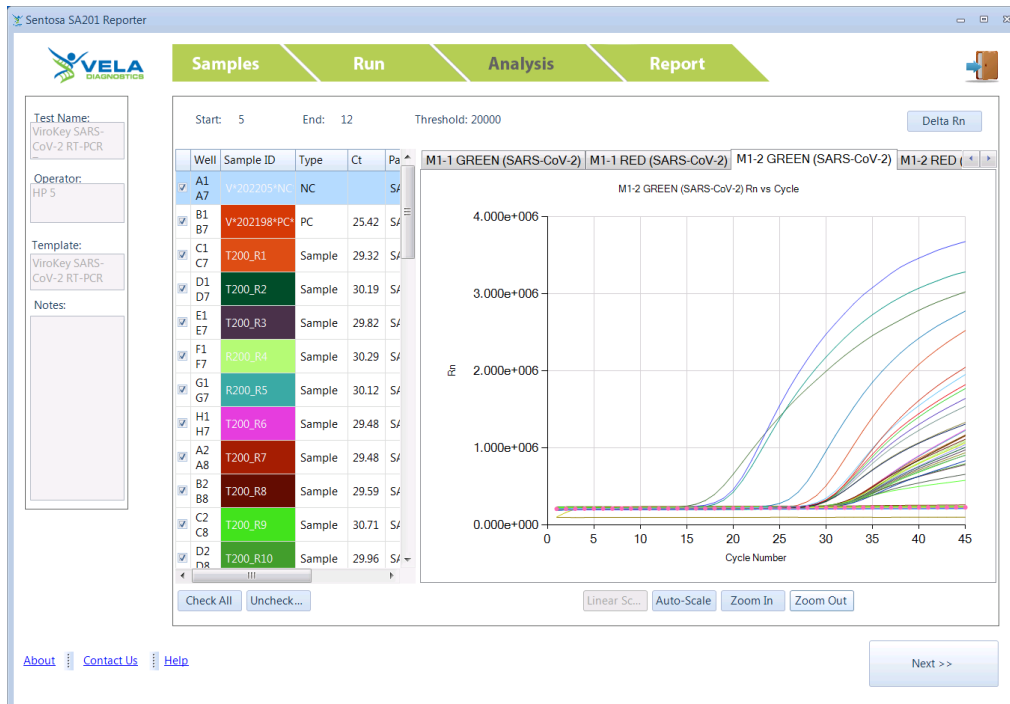
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The *Sentosa*® SA201 Series Software may now be closed by clicking the close button “” on the title bar. On the *Sentosa*® SA201 Reporter, click “*Next*” to proceed to data analysis.

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4. 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.


4.1. Report generation

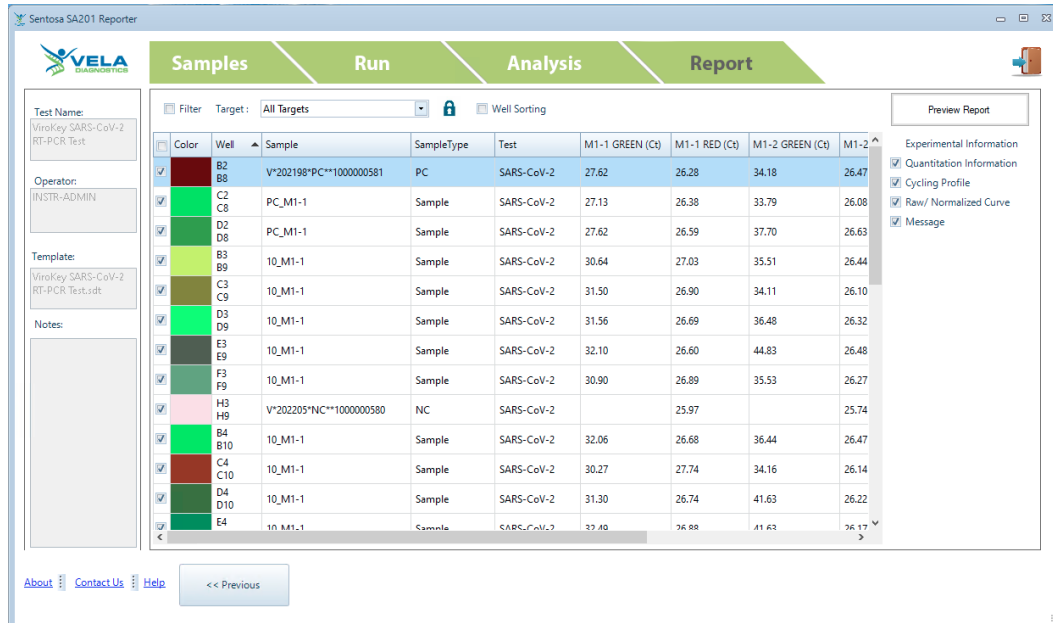
Under “Report” tab, there are other functions available as mentioned below:

- Activate “Filter” checkbox to display the selected samples, or deactivate “Filter” checkbox to hide the selected samples. User can also select

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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 C_t*” column will display the C_t value for the samples.

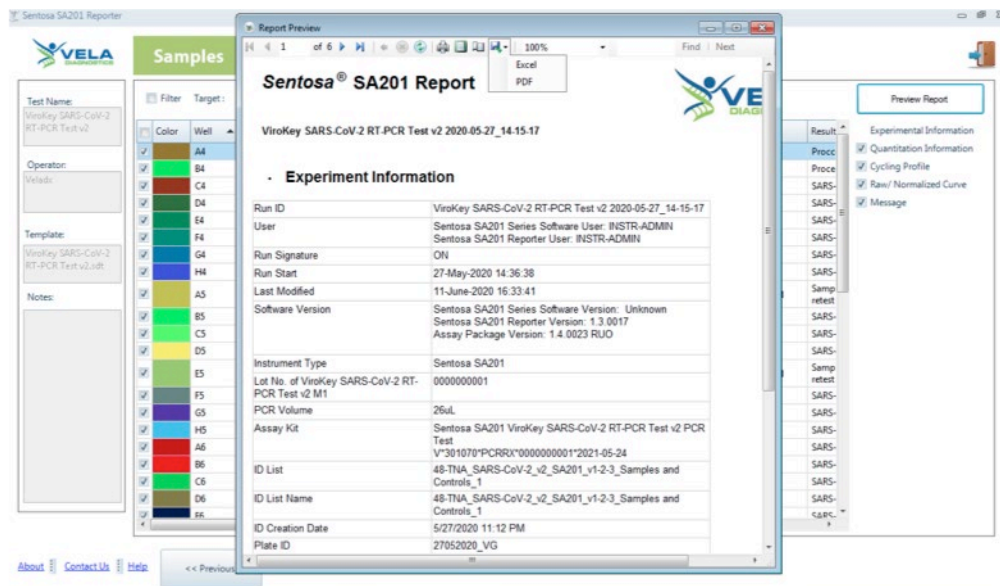


The screenshot shows the Sentosa SA201 Reporter software interface. The main window displays a table of sample data. The table has columns for Color, Well, Sample, SampleType, Test, and four fluorescence channels (M1-1 GREEN (Ct), M1-1 RED (Ct), M1-2 GREEN (Ct), M1-2). The table is filtered to show all targets. On the right, there is a 'Preview Report' button and a section for 'Experimental Information' with checkboxes for Quantitation Information, Cycling Profile, Raw/Normalized Curve, and Message.

Color	Well	Sample	SampleType	Test	M1-1 GREEN (Ct)	M1-1 RED (Ct)	M1-2 GREEN (Ct)	M1-2
B2	B8	V*202198*PC**1000000581	PC	SARS-CoV-2	27.62	26.28	34.18	26.47
C2	C8	PC_M1-1	Sample	SARS-CoV-2	27.13	26.38	33.79	26.08
D2	D8	PC_M1-1	Sample	SARS-CoV-2	27.62	26.59	37.70	26.63
B3	B9	10_M1-1	Sample	SARS-CoV-2	30.64	27.03	35.51	26.44
C3	C9	10_M1-1	Sample	SARS-CoV-2	31.50	26.90	34.11	26.10
D3	D9	10_M1-1	Sample	SARS-CoV-2	31.56	26.69	36.48	26.32
E3	E9	10_M1-1	Sample	SARS-CoV-2	32.10	26.60	44.83	26.48
F3	F9	10_M1-1	Sample	SARS-CoV-2	30.90	26.89	35.53	26.27
H3	H9	V*202205*NC**1000000590	NC	SARS-CoV-2		25.97		25.74
B4	B10	10_M1-1	Sample	SARS-CoV-2	32.06	26.68	36.44	26.47
C4	C10	10_M1-1	Sample	SARS-CoV-2	30.27	27.74	34.16	26.14
D4	D10	10_M1-1	Sample	SARS-CoV-2	31.30	26.74	41.63	26.22
E4	E10	10_M1-1	Sample	SARS-CoV-2	32.49	26.88	41.63	26.17

Click “*Preview Report*” to preview the report.

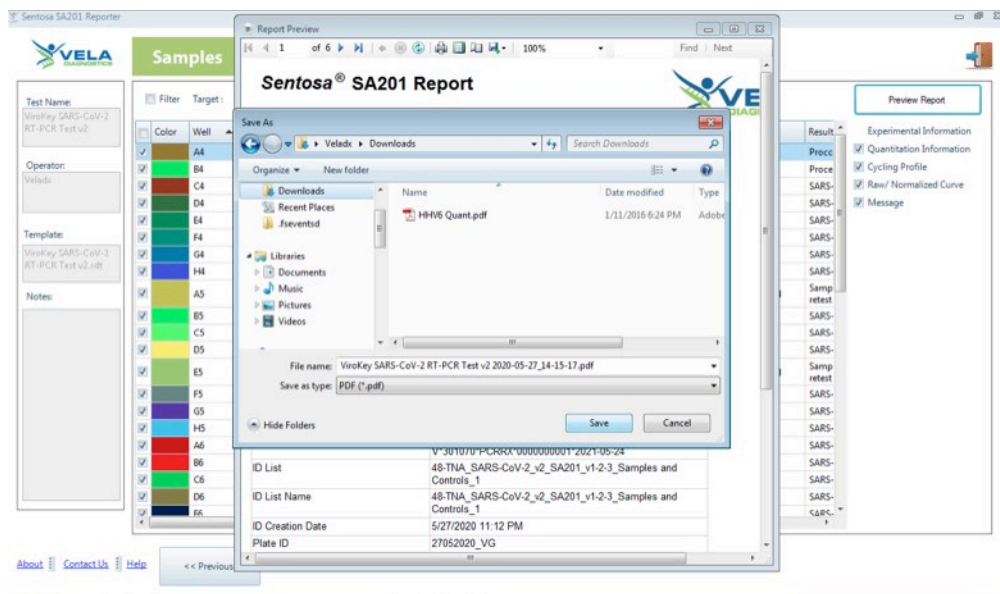
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4.2. The “Report Preview” pop-up window will be displayed. The report can be saved, printed and / or exported to PDF or Excel.

4.3. 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 after data analysis” on page 10.

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


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

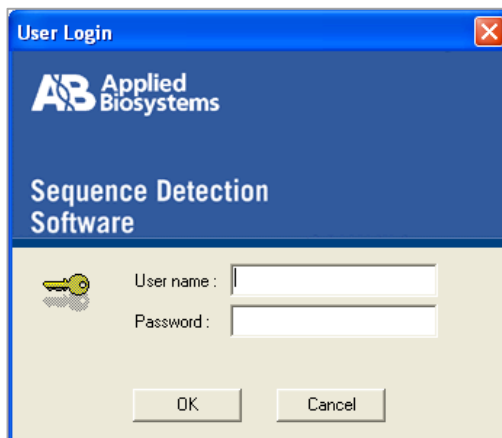
- 5.1. Turn on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System by pressing the power button on the instrument, and wait for the initiation procedure to be completed.

NOTE: Ensure the green indicator is lit and not flashing.



- 5.2. Run Reverse Transcription PCR

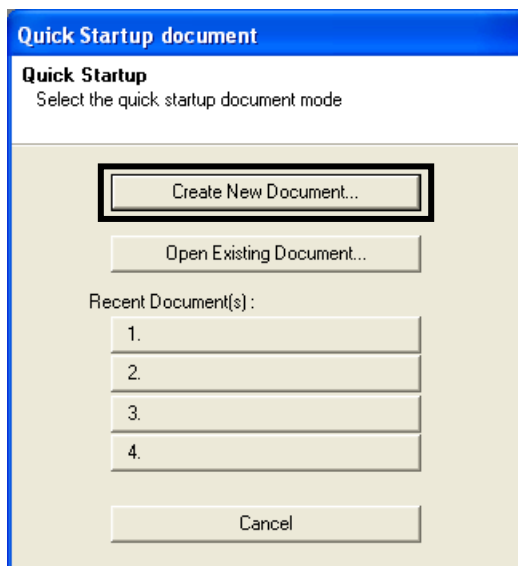
- 5.2.1. On the instrument's computer, launch the ABI 7500 Fast System SDS Software by double-clicking the  icon. The “Login” window opens, type the account name and password, and then click on “OK”.



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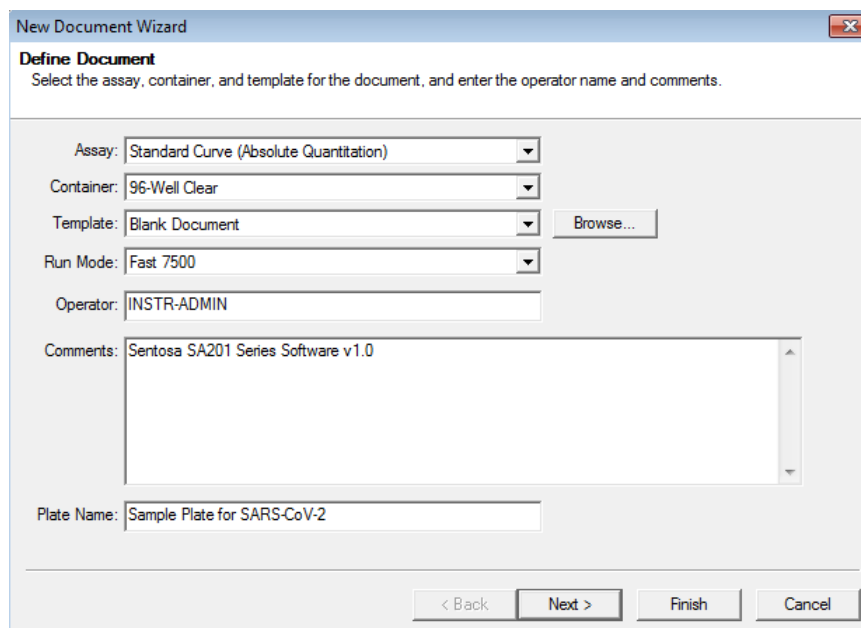
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- 5.2.2. In the “Quick Startup document” dialog box, select “Create New Document”.



- 5.2.3. 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”.



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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.

The screenshot shows a 'New Document Wizard' dialog box with the 'Define Document' step selected. The dialog box has a title bar with a close button. Below the title bar, the text 'Define Document' is followed by the instruction 'Select the assay, container, and template for the document, and enter the operator name and comments.' The form contains several fields: 'Assay' is a dropdown menu set to 'Standard Curve (Absolute Quantitation)'; 'Container' is a dropdown menu set to '96-Well Clear'; 'Template' is a dropdown menu set to 'ViroKey SARS-CoV-2 v2.sdt' with a 'Browse...' button to its right; 'Run Mode' is a dropdown menu set to 'Fast 7500'; 'Operator' is a text field containing 'veladx'; 'Comments' is a large text area containing 'Sentosa SA201 Series Software v1.0.1'; and 'Plate Name' is a text field containing 'Sample Plate v2'. At the bottom of the dialog box are four buttons: '< Back', 'Next >', 'Finish' (which is highlighted with a black border), and 'Cancel'.

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- 5.2.4. 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.

New Detector

Name: Green - ORF1a

Description:

Reporter Dye: FAM

Quencher Dye: (none)

Color:

Notes:

Create Another OK Cancel

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- 5.2.5. 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*”.

New Document Wizard

Select Detectors
Select the detectors you will be using in the document.

Find: ▲ ▼

Detector Name	Description	Reporter	Quencher
Green - ORF1a		FAM	(none)
Orange - N		VIC	(none)
Red - EC		CY5	(none)

Add >>
<< Remove

Passive Reference: ROX ▼

Detectors in Document
Green - ORF1a
Orange - N
Red - EC

New Detector...

< Back **Next >** Finish Cancel

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- 5.2.6. 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.

New Document Wizard ✕

Set Up Sample Plate
Setup the sample plate with tasks, quantities and detectors.

Use	Detector	Reporter	Quencher	Task	Quantity
<input checked="" type="checkbox"/>	Green - ORF1a	FAM	(none)	Unknown	
<input checked="" type="checkbox"/>	Orange - N	VIC	(none)	Unknown	
<input checked="" type="checkbox"/>	Red - EC	CY5	(none)	Unknown	

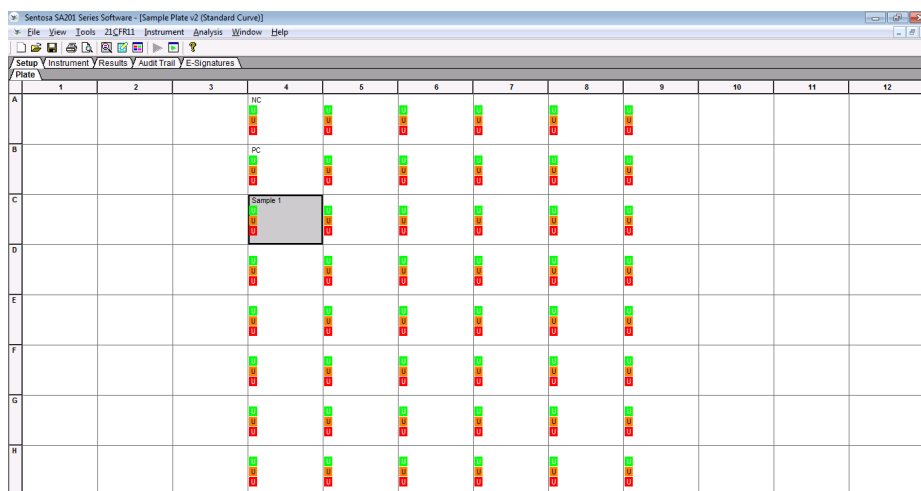
<
>

	1	2	3	4	5	6	7	8	9	10	11	12
A				U U U	U U U	U U U	U U U	U U U	U U U			
B				U U U	U U U	U U U	U U U	U U U	U U U			
C				U U U	U U U	U U U	U U U	U U U	U U U			
D				U U U	U U U	U U U	U U U	U U U	U U U			
E				U U U	U U U	U U U	U U U	U U U	U U U			
F				U U U	U U U	U U U	U U U	U U U	U U U			
G				U U U	U U U	U U U	U U U	U U U	U U U			
H				U U U	U U U	U U U	U U U	U U U	U U U			

< Back
Next >
Finish
Cancel

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- 5.2.7. 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).

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5.2.8. 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.

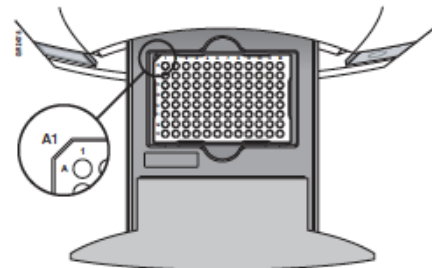
The screenshot shows the ViroKey instrument software interface. The 'Instrument' tab is selected. The 'Thermal Cycler Protocol' section displays a graph of the thermal profile. The graph shows two stages: Stage 1 and Stage 2. Stage 1 has a temperature of 50.0°C for 15:00 and 95.0°C for 3:00. Stage 2 has a temperature of 95.0°C for 0:15 and 58.0°C for 0:30. The 'Settings' section at the bottom shows 'Sample Volume (µL)' set to 26, 'Run Mode' set to 'Fast 7500', and 'Data Collection' set to 'Stage 2, Step 2 (58.0 @ 0:30)'.

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)*”.

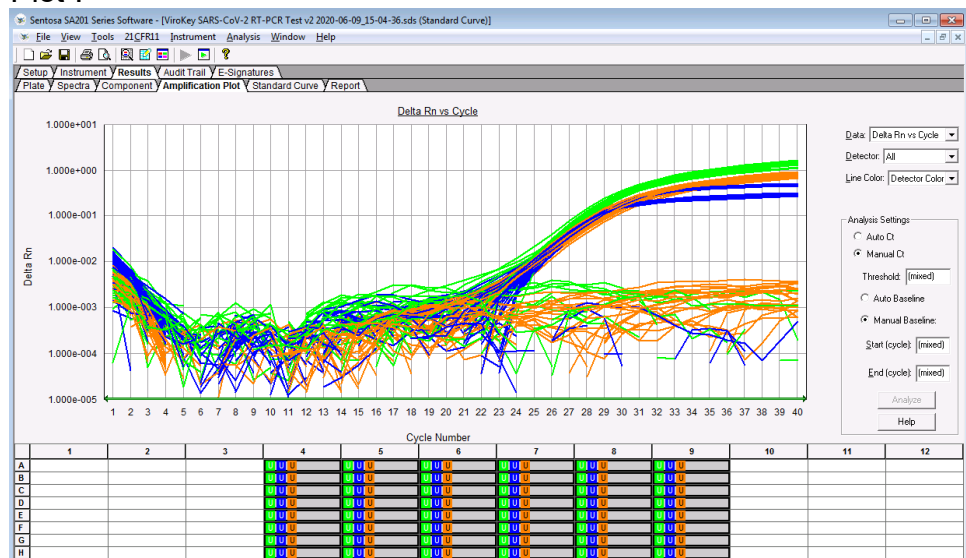
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- 5.2.9. Save the run file in “sds” format. The plate document can also be saved as a template document for future use. To do this, save the template file in “sdt” format in a preferred folder.
- 5.2.10. At the front of the Applied Biosystems® 7500 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.

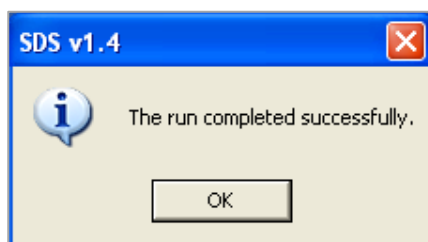


- 5.2.11. Under “Instrument Control” on the “Instrument” tab, click “Start”.
- 5.2.12. Information on real-time status of the run based on fluorescence emissions is displayed in the “Results” tab, under “Amplification Plot”.



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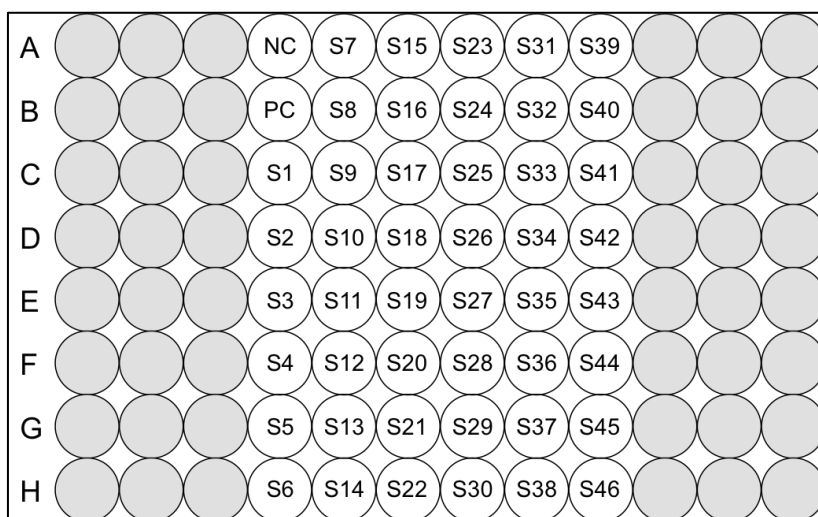
- 5.2.13. At the end of the run, a message appears indicating whether or not the run is successful. All data generated is saved in the “.sds” file specified in step 5.2.9.



- 5.2.14. After the run is completed, proceed to section 6, “Data analysis on the Applied Biosystems® 7500 Fast Dx Real-Time PCR System”.

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

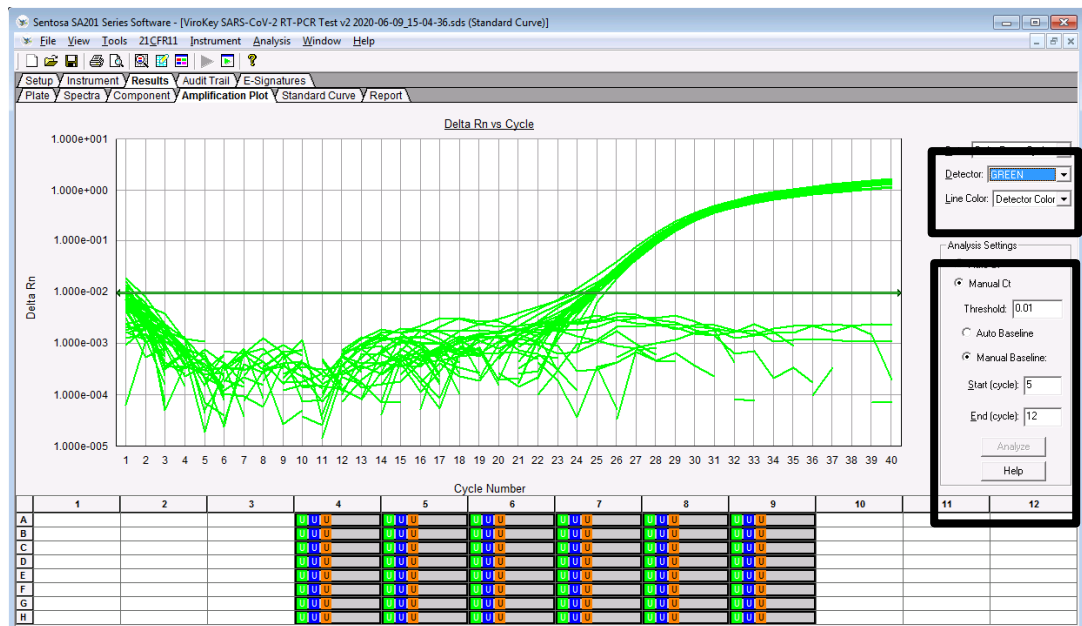
For the automated workflow runs, please refer to the plate layout below and analyze the results accordingly.



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

- 6.1. 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*”).

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

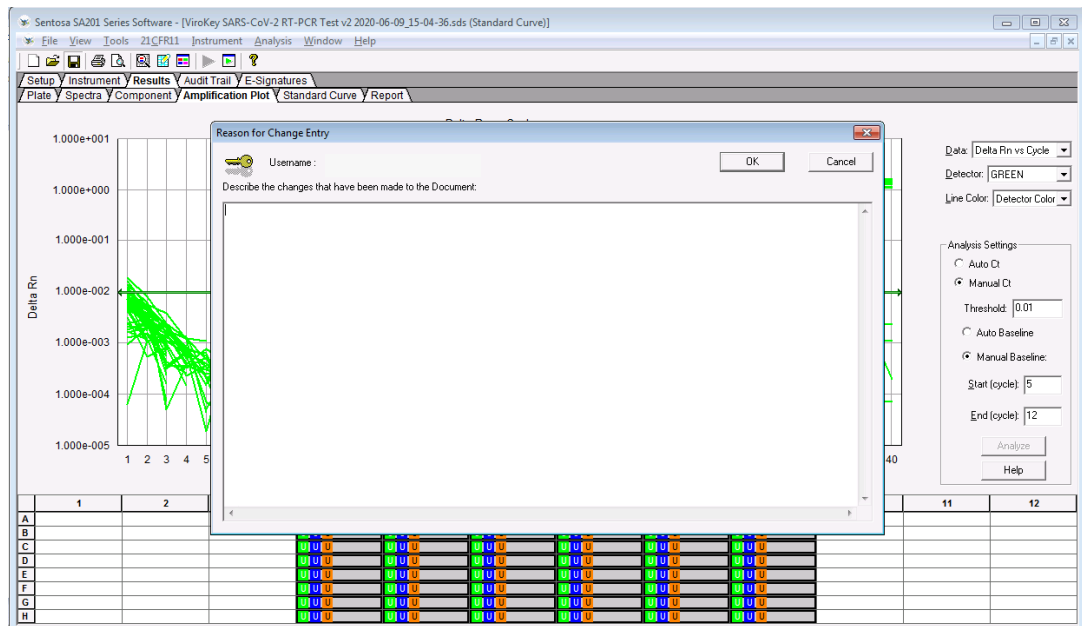
Detector	Threshold	Baseline	
		Start (cycle)	End (cycle)
Green – ORF1a	0.01	5	12
Orange - N	0.007	5	12
Red EC	0.01	5	12

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.

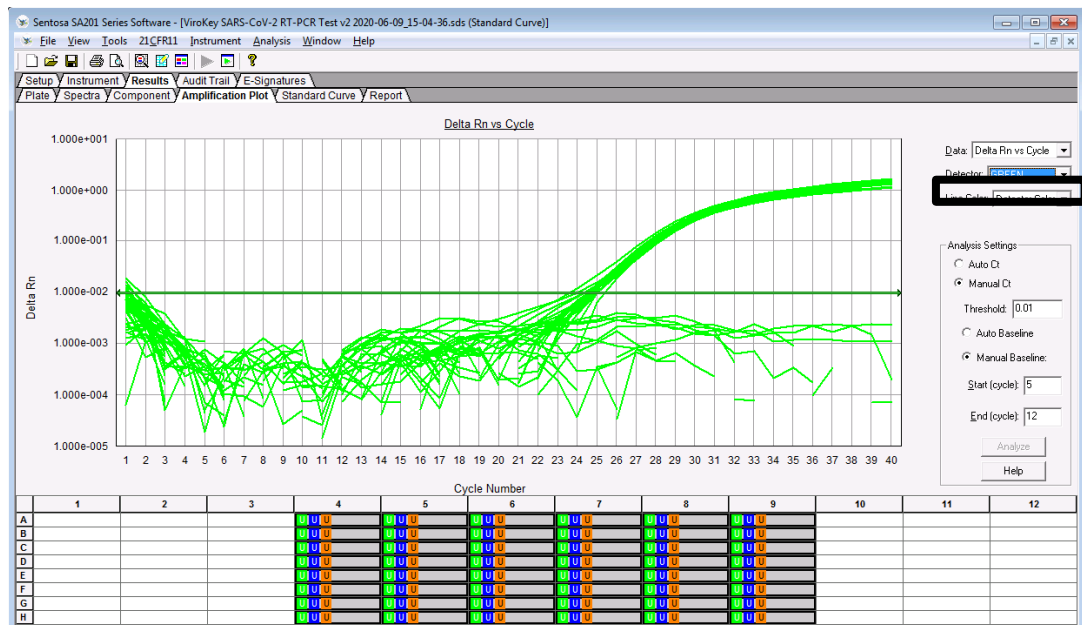
Repeat these steps to set the threshold and baseline values for all detectors.

- 6.2. 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.

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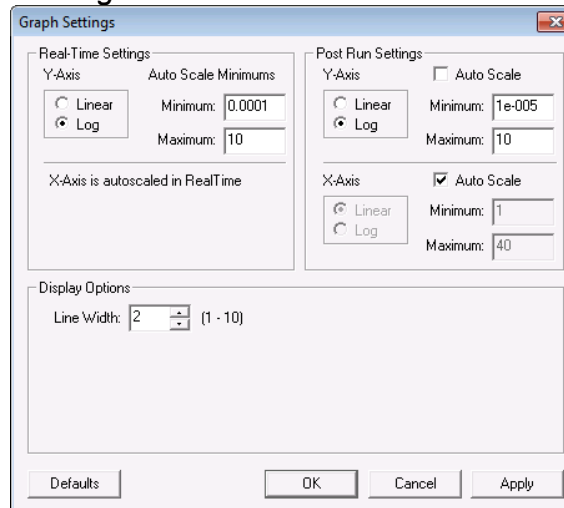
- 6.3. To generate graphs for specific samples or target channel, select the corresponding sample wells at the bottom of the window, then choose the desired target channel from the "Detector" drop-down list. Select "GREEN" for the analysis of the SARS-CoV-2 target ORF1a, "ORANGE" for the analysis of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



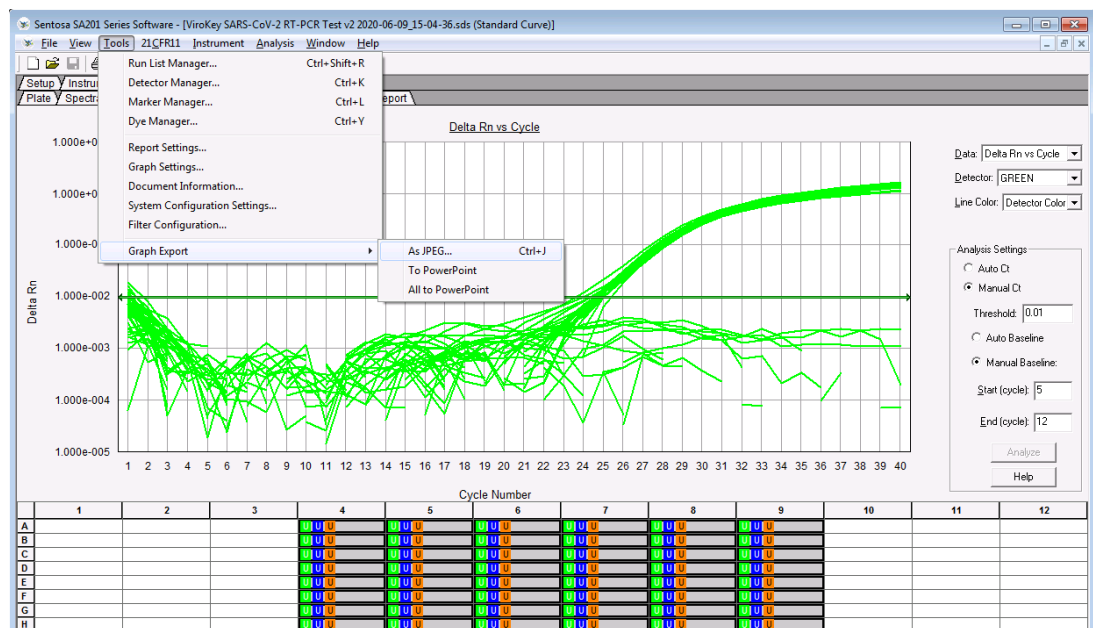
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Right click on the graph to select “*Graph setting*”. In “*Post Run setting*” category, choose “*Log*” for Y-axis.

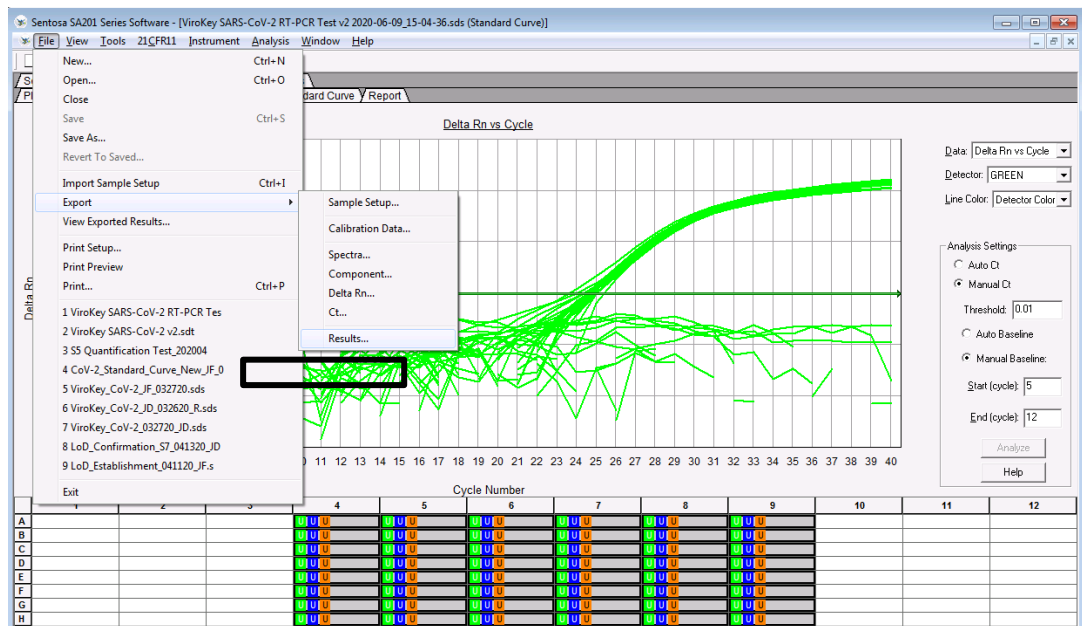


- 6.4. To export the graphs for the run, select “*Tools*” menu -> “*Graph Export*”, then choose the desired format to export the graph in.

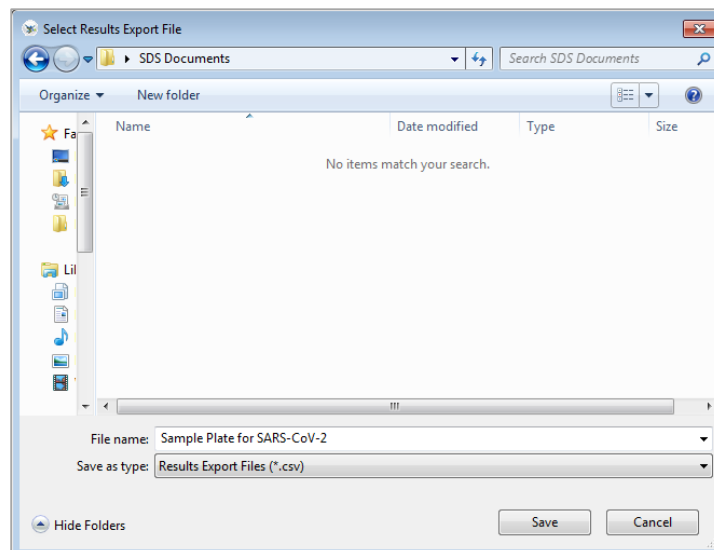


- 6.5. To export results, save the “.sds” file first. Select “*File*” menu -> “*Export*”, then choose the desired information to export.

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- 6.6. 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 after data analysis” on page **Error! Bookmark not defined.**

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 *Sentosa*® SX101 and the *Sentosa*® SA201 after each run. Refer to the respective instrument user manuals 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

- | | |
|------------------------|--|
| a) Storage of reagents | Reagents might precipitate upon storage. If required, shake vigorously to dissolve the precipitates. |
|------------------------|--|

3. Consistent high C_T 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 <i>Sentosa</i> ® 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

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a) PCR conditions do not comply with the protocol	Ensure that the correct thermal cycling conditions are input into the <i>Sentosa</i> ® 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 <i>Sentosa</i> ® SA201 Series 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

a) PCR conditions do not comply with the protocol	Ensure that the correct thermal cycling conditions are input into the <i>Sentosa</i> ® SA201 Series 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 <i>Sentosa</i> ® 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.

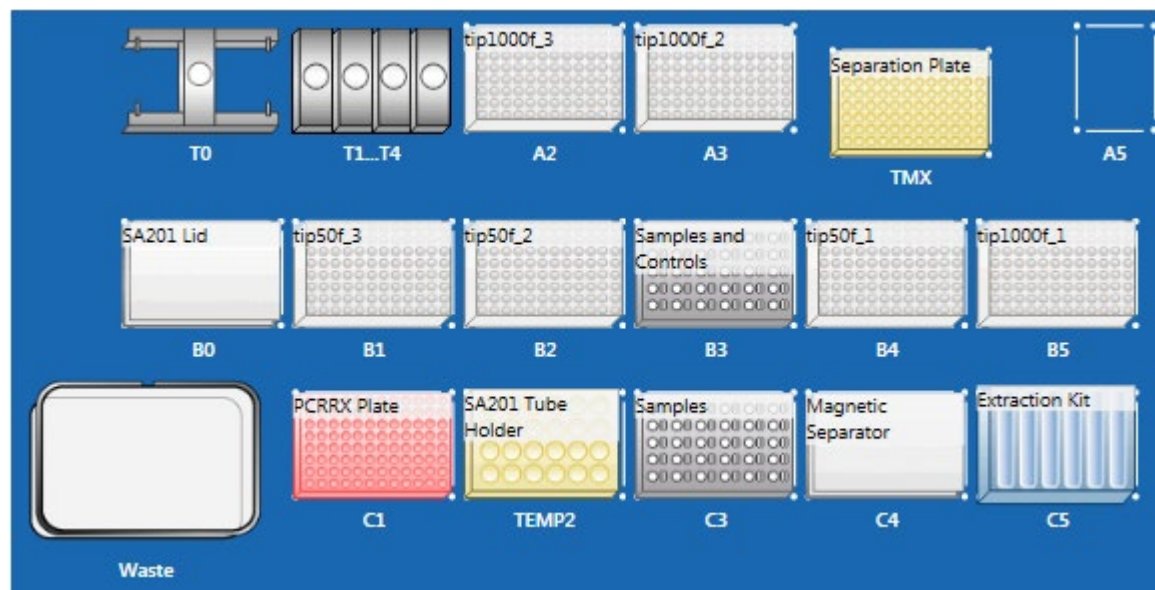
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e) Storage conditions for one or more kit components did not comply with the instructions given in the "Storage" section	Check the storage condition (refer to the kit label) of the reagents and use a new kit if necessary.
f) Extraction / assay kit 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	<p>Repeat the extraction and PCR protocols with new reagents.</p> <p>Ensure that the workspace and instruments are decontaminated as recommended.</p> <p>First check the Rn for true amplification profile and rule out baseline issues.</p>
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 A: *Sentosa*[®] SX101 layout for 48-1 ViroKey SARS-CoV-2-v2 v3-3 application



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

References

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2020-07-02.

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For *in vitro* diagnostic (IVD) use



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

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 (4x50) on an automated workflow using the liquid handler Sentosa® SX 101 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 Blue 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 M2 and 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 (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

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 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 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 is an enzyme, which is in liquid state. Except RNA4 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 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 immediately after use at ≤ -20°C for subsequent reactions.
- All reagents, except RNA4 M2 and RNA4 M3, require thorough mixing by quick vortex. Mix RNA4 M2 and RNA4 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.

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
	Use-by date
	For <i>in vitro</i> diagnostic (IVD) use
	Prescription device
	Catalog number
	Component
	Number
	Content
	Lot number
	Control
	Negative control
	Positive control
	Document / label identification number
	Temperature limitations
	Legal manufacturer

Symbol	Description
	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 and ViroKey™ SX Virus Total Nucleic Acid Kit (4x48) are available for download at www.veladx.com by logging in as an authorized user, or requesting them via email.

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2021-07-07.

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