User's Guide

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Kaira 2019-nCoV Detection Kit

IVD

REF RDM101-X

For Emergency Use Authorization (EUA) Only

For *in vitro* Diagnostic (IVD) Use

For Prescription Use Only

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Safety Warnings and Precautions

Please inquire OPTOLANE's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.
Please read the User's Guide and check the integrity of all tubes, tips and other materials supplied with this kit prior to use.
Before, during and after use of this kit as described in this User's Guide, all potentially hazardous materials (i.e. materials that may have come in contact with clinical samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/ government in which this product is being used. Adhere to general clinical laboratory safety procedures during the experiment.

Warranty and Liability

All OPTOLANE's products are manufactured and tested under strict quality control protocols. OPTOLANE's guarantees the quality of all directly manufactured products until the expiration date printed on the label. If any issues are discovered relating to compromise in product quality, immediately contact OPTOLANE's Customer Service Center.
OPTOLANE does not assume liability for misuse of the product, i.e. usage of the product for any purposes other than its intended purpose as described in the appropriate and applicable User's Guide. OPTOLANE assumes liability under the condition that the user discloses all information related to the problem to OPTOLANE in written form within 30 days of occurrence.

Legal Disclaimer

The use of the kit is only for qualified and well-trained users in handling of clinical specimens and molecular biological experiments. After testing, all waste should be processed with the fulfillment of the regulation of the country.

TABLE OF CONTENTS

1. INTENDED USE

Kaira 2019-nCoV Detection Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab specimens, as well as nasopharyngeal wash/aspirate, nasal aspirate, sputum and bronchoalveolar lavage (BAL) specimens 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 which is generally detected in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2; 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.

Testing with the *Kaira* 2019-nCoV Detection Kit 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. *Kaira* 2019-nCoV Detection Kit is intended for use only under the Food and Drug Administration's Emergency Use Authorization.

2. INTRODUCTION

Coronavirus (CoV) is a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). SARS-CoV2 (2019-nCoV), the cause of COVID-19, is a new strain that was discovered in 2019 and has not been previously identified in humans. Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans. Several known coronaviruses are circulating in animals that have not yet infected humans. To date, there is no vaccine and no specific antiviral medicine to prevent or treat SARS-CoV-2 infection-19. The only treatment for people affected is to relieve symptoms. Therefore, people with illness should be hospitalized. Currently, some specific drug treatments are under investigation which is being tested through clinical trials.

Molecular test is one of the most effective way to diagnose the presence of SARS-CoV-2 in people who are thought to be infected. In particular rRT-PCR-assays are molecular tests that can be used to detect viral RNA in clinical samples. OPTOLANE Technologies Inc. introduces *Kaira* 2019-nCoV Detection Kit to detect SARS-CoV-2 in human samples through-rRT-PCR.

3. FEATURES AND PRINCIPLE OF THE TEST

Real-time PCR involves the selective amplification of a target sequence while monitoring the progress of amplification in real-time through a visualizing agent such as a fluorescent dye. The specificity is provided by a pair of specific primers, along with a hydrolysis probe which is also sequence specific. Monitoring amplified product is conducted by labeling the hydrolysis probe with a matched pair of fluorescent dyes (5'-Fluorescent reporter; 3'- Quencher). However, upon cleavage by the 5' – 3' exonuclease activity of the DNA polymerase during PCR, the fluorescent reporter molecule will emit a specific wavelength of light within the visible spectrum when cleaved after binding to the amplicon.

4. CONTENTS AND RELATED INSTRUMENTS

4.1. Contents of the Kit

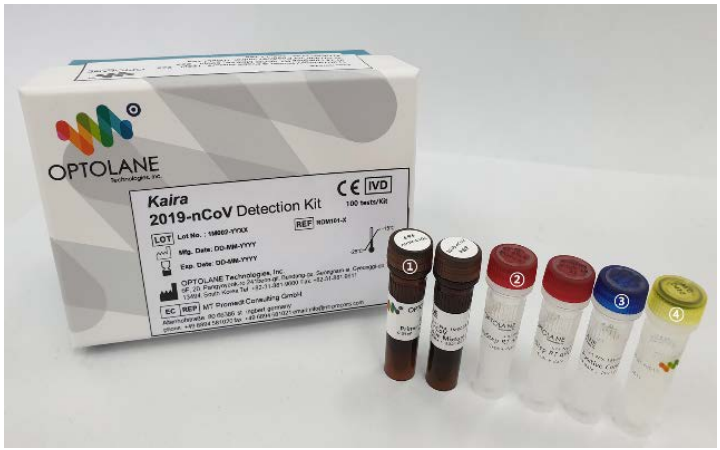


Figure 1. *Kaira* 2019-nCoV Detection Kit (100 tests/kit)

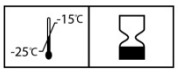
Table 1. Components of *Kaira* 2019-nCoV Detection Kit

Reagents		Contents	Unit	Quantity
①	2019 nCoV Primer & Probe Mixture	Rdrp and E gene specific primers and probe, PCRC (Cpn21) specific primers and probe, PCRC (Cpn21) plasmid DNA, Water	125μL/tube	2 tube
②	2× OneStep RT qPCR Mixture	Buffer containing One-step RT Realtime PCR Enzyme Mix (Hot-start Taq and Reverse Transcriptase), KCl, NaCl, and Water	625μL/tube	2 tube
③	2019 nCoV Positive control DNA	RdRP and E gene specific DNA, Water	100μL/tube	1 tube
④	DEPC-treated water	Water	200μL/tube	1 tube

4.2. PCR Instruments

This kit is optimized and validated for use with QuantStudio 5 Real-Time PCR system (Thermo-Fisher Scientific; Software ver. 1.5.1), ABI 7500 Real-Time PCR system (Applied Biosystems co.; Software ver. 2.3), or CFX96 Dx System (Bio-Rad Laboratories; Software ver. 3.1.1517.0823). For detailed operating instructions of ThermoFisher Scientific QuantStudio 5 Real-Time PCR, ABI 7500 Real-Time PCR systems, or Bio-Rad CFX96 Dx system, please refer to the instrument *User's Guide*.

5. STORAGE AND EXPIRATION DATE



The *Kaira* 2019-nCoV Detection Kit should be stored at -25ºC to -15ºC away from UV/sunlight. The kit is guaranteed stable until the expiration date printed on the label. Repeated thawing and freezing (more than four times) of the components should be avoided, as this may reduce assay performance. Do not use reagents past their expiration date.

6. REQUIRED MATERIALS AND EQUIPMENT (NOT PROVIDED)

- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Clean bench
- Cooling device or ice
- Desktop centrifuge (1.5 mL microcentrifuge and 96 well plate centrifuge)
- Vortex mixer
- For Bio-Rad CFX96 system: Low-Profile PCR tubes 8-tube strip, white (Cat. TLS0851) and Optical Flat 8-cap Strips for 0.2 ml tube strips/plates (Cat. TCS0803)
- For ABI QS5 and 7500 system: MicroAmp Optical 8-tube strip (0.2mL) (Cat. 4316567) and MicroAmp Optical 8-Cap Strip (Cat. 4323032)
- For automated nucleic acid extraction: QIAsymphony SP (QIAGEN, Cat. 9001297, Germany) with its add-on kit QIAsymphony DSP Virus/Pathogen Kit (QIAGEN, Cat. 937036, Germany).

7. GENERAL PRECAUTIONS

7.1. Warning

- For in vitro diagnostic use (IVD) only.
- For Emergency Use Authorization only.
- For Prescription Use only.
- The *Kaira* 2019-nCoV Detection Kit has not been FDA cleared or approved.
- The *Kaira* 2019-nCoV Detection Kit 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 *Kaira* 2019-nCoV Detection Kit has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The *Kaira* 2019-nCoV Detection Kit is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics 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.
- Real-Time PCR through this kit should be performed using QuantStudio 5 Real-Time PCR system, ABI 7500 Real-Time PCR system, or CFX96 Dx system.

- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.
- Always wear gloves and a mask when handling specimens and kit reagent.
- DO NOT mix reagents from different production lots.
- DO NOT use a kit after its expiration date.
- This kit is for use only with specimens indicated in the intended use.
- Avoid microbial contamination of kit components when preparation of specimens.
- Please read this *User's Guide* before use.
- DO NOT change the protocol as described in this *User's Guide*.
- Always use sterile, filtered pipette tips.
- Clinical samples and their derivatives should be stored in a separate location/freezer from where the rest of the kit components are stored.
- Briefly vortex and spin-down all kit components after thawing to ensure optimum results.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- Positive results are indicative of the presence of SARS-CoV-2. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

8. PROTOCOL

8.1. Preparation

We recommend that several precautionary measures be taken for the safety of user and laboratory and for the prevention of laboratory environmental contamination.

8.1.1 Proper Use of a Biosafety Cabinet

When handling clinical samples, all related works (i.e. decapping, pipetting, capping of clinical samples and containers) should be conducted within a negative pressure biosafety cabinet (Class II). Negative pressure biosafety cabinet sends air from the laboratory space outside. In other words, air flows inward. This airflow prevents dangerous substances from contaminating the laboratory environment. Positive pressure biosafety cabinet is a workspace where filtered air flows outward, thus keeping a clean environment within the workspace.

8.2. Specimen

All samples should be treated as potential biohazards.

8.2.1 Specimen Collection, Handling, Testing, Storage, and Transport

Collect nasopharyngeal swab (NP), oropharyngeal swab (OP), anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, bronchoalveolar lavage (BAL) specimens, and sputum according to CDC guidelines and manufacturer's protocol for sample collection, storage, and handling. Individuals who are collecting specimens or within 6 feet of patients suspected to be infected with SARS-CoV-2 should maintain proper infection control based on the CDC guideline. CDC recommends using personal protective equipment, which includes an N95 or higher-level respirator (or facemask if a respirator is not available), gloves, eye protection, and a gown. For individuals who are handling specimens but are not directly involved in collection (e.g. self-collection) and not working within 6 feet of the patient, please refer to standard precautions as CDC recommended. After collection, the specimen should be stored at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, specimens should be stored at -70°C or below. If delivery and processing exceed 72 hours, specimens should be transported in dry ice and once in laboratory frozen at -70°C or colder. Once the specimens have been collected according to the CDC guideline, it is recommended to use Universal Transport Medium (UTM, Copan diagnostics, Cat. 305C, USA) for transportation/ temporary storage.

For more details, please refer to CDC guidelines for sample collection and storage at:
https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html

Sample transportation: Specimens must be packaged and shipped in accordance with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations and Guidance of the Centers for Disease Control and Prevention (CDC).

8.3. Procedure

8.3.1 Workflow

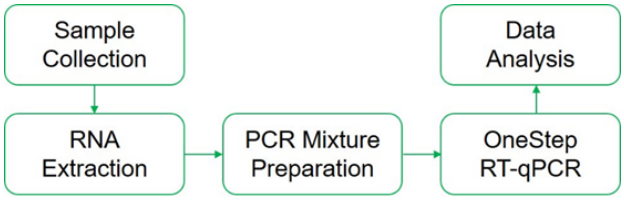


Figure 2. Example of workflow for the detection of SARS-CoV-2

Schematic workflow for detecting 2019 nCoV is shown in figure 2.

8.3.2 Nucleic Acid Extraction

Nucleic acid (RNA) is performed using the automated extraction instrument, QIAsymphony SP (Qiagen, Cat. 9001297) and the extraction kit, QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Cat. 937036). For extraction, the initial specimen test volume is 200µL and the final elution volume is 60 µL. For additional instructions, please refer to the instructions for use for the QIAsymphony SP instrument and QIAsymphony DSP Virus Pathogen Mini Kit. It is recommended that external positive and negative extraction controls are included in each extraction run (See Quality Control Section below).

If real-time RT-PCR is not conducted immediately after nucleic acid extraction, RNA should be stored at -20 °C until use. For long term storage, RNA samples should be stored at -70°C or below.

8.3.3 PCR Preparation

1) The NTC and PC kit controls, as well specimen RNA extracts and external positive and negative control extracts should be thawed for at least 10 minutes at room temperature (if frozen).
2) Vortex more than 10 seconds and briefly spin-down all reagents, controls and samples prior to use.
3) Calculate the amount of the PCR mastermix required for the test based on the number of clinical specimens and controls (See section 8.3.5 below) as well as an extra two additional reactions to compensate for some loss. Prepare a PCR mastermix by mixing 2x OneStep RT-qPCR mixture with 2019-nCoV Primer & Probe Mixture. Briefly, for one reaction, the PCR mastermix will contain 12.5 µL of 2x OneStep RT-qPCR Mixture and 2.5µL of 2019-nCoV Primer & Probe Mixture for a total of 15 µL of prepared PCR mastermix per PCR reaction (See Table 2). Vortex and spin-down the PCR mastermix for more than 10 seconds before use.

Table 2. Components and volumes of reaction mixture

Component	Volume (µL)
2× OneStep RT-qPCR Mixture	12.5
2019-nCoV Primer & Probe Mixture	2.5
RNA specimen, PC, or NTC	10
Total PCR reaction volume	**25**

4) Add 15 µL of prepared mastermix to all assigned PCR wells.
5) Add 10 µL of DEPC-treated water into the tube assigned for NTC. Please seal the tubes with cap to prevent contamination from other tubes.
6) Move the tubes to a separated location before proceeding to prevent contamination. Add 10 µL of prepared low-positive PC to the tube assigned for PC (Please refer to Section below for preparation of PC).
7) Add 10 µL of extracted nucleic acids of clinical samples and external controls to tubes assigned.
8) Seal the tubes with cap completely.
Note: In order to avoid contamination and invalid results, seal all the tubes accordingly.
9) To mix the tubes thoroughly, vortex for 5 sec and centrifuge at 1500 rpm for 2 min.
10) Place the mixed tubes in the QuantStudio 5 Real-Time PCR system, ABI 7500 Real-Time PCR system, or CFX96 Dx system and run PCR immediately (Please refer to each instrument's *User's Guide*).

8.3.4 OneStep RT-qPCR setting

For instrument software setting, please refer to the user manual for each instrument. Table 3 represents threshold values for each instrument. For adjustment of thresholds in each instrument, please refer to Appendix 1. The PCR conditions for testing with the *Kaira* 2019-nCoV Detection Kit are presented in table 4.

Table 3. Criteria for threshold values by PCR instruments

PCR machine	Target	E-gene	RdRp	PCRC
QS5	Max ΔRn	1,000,000	1,000,000	300,000
	Threshold	20,000	50,000	20,000
ABI7500	Max ΔRn	1,000,000	1,000,000	300,000
	Threshold	20,000	50,000	20,000
CFX96	Max ΔRn	21,000	10,000	2,000
	Threshold	1,000	500	300

Table 4. PCR conditions for *Kaira* 2019-nCoV detection kit

STEP	Temperature	Running Time	Cycle Number
Reverse transcription	50°C	10 minutes	1 cycle
Pre-Denaturation	95°C	10 minutes	1 cycle
Denaturation	95°C	10 seconds	45 cycles
Annealing & Extension *	60°C	30 seconds	
END			

* This step scanned the fluorescence signal.

8.3.5 Quality Control/Assay Controls:

Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. Quality Control procedures are intended to monitor reagent and assay performance.

- Positive Control (PC): The positive control is comprised of non-infectious DNA plasmids containing RdRp gene and E gene fragments used to verify PCR amplification process and is used in every test. The Positive Control should be diluted to a concentration of ~5x LoD (see instructions below) and should yield a positive result for each target in the *Kaira* 2019-nCoV Detection Kit.
- No Template Control (NTC): NTC is used as a PCR control to confirm test validity, and the absence of any contaminants during testing. The "No template" control is prepared using DNase/RNase-free Water added to the Master Mix prior to PCR. NTC must be included in each test run. The NTC should yield negative results for the RdRp and E gene targets and a positive result for the internal control (PCRC).
- Internal Control (PCRC): The *Kaira* 2019-nCoV Detection Kit uses the CPN21 plasmid DNA as an exogenous control which is included along with the primers/probe for the CPN21 in the 2019 nCoV Primer & Probe Mixture. The CPN21 gene internal control (PCRC) is used to monitor real time PCR amplification process.
- External Negative Extraction Control (NC): It is recommended that a negative extraction control consisting of DNase/RNase free water is included in each extraction batch. The NC is used to monitor for potential contamination during extraction and assay preparation. The NC should yield a negative result for the RdRp and E gene targets.
- External Positive Extraction Control: The *Kaira* 2019-nCoV Detection Kit does not include an internal control for RNA extraction/recovery or reverse transcription. A known SARS-CoV-2 positive sample or sample containing SARS-CoV-2 RNA (e.g., *in vitro* transcript or pseudovirus) should be tested with every batch of patient specimens to monitor the integrity of the extraction and reverse transcription (See Section 8.3.6 below for additional information).
- Additional controls should be tested in accordance with state and institutional guidelines and accreditation requirements.

[Preparation of positive control]

The Positive Control (PC) includes approximately 1,500,000 copies/mL and 1,000,000 copies/mL for the RdRp and E gene targets respectively. The PC should be diluted to concentration that contains approximately 10,000 copies/mL and 5,000 copies/mL for the RdRp And E gene targets.

The following shows brief methods for the preparation of final positive control.
1) Dilute Positive kit control (PC) to 1:60 with 1x PBS (i.e. put 1 µL of PC into 59 µL of 1x PBS).
2) Take 10 µL of the diluted PC and mix with 15 µL of mastermix (2x OneStep RT-qPCR Mixture with 2.5µL of 2019-nCoV Primer & Probe Mixture).
3) Run PCR according to the instruction. When the amplification is "detected", it is considered that the detection system for SARS-CoV-2 is effective.

8.3.6 Application of external positive control

The *Kaira* 2019-nCoV Detection Kit does not include an internal control for RNA extraction/recovery or reverse transcription. A known SARS-CoV-2 positive sample or sample containing SARS-CoV-2 RNA (*in vitro* transcript or pseudovirus) should be tested with every batch of patient specimen to monitor the integrity of these process steps. As an example, 200 µL of encapsulated synthetic RNA (SeraCare; AccuPlex™ SARS-CoV-2 Verification Panel V2, (Positive 1: 100,000 copies/mL) (#0505-0132)) was validated with the Kaira 2019-nCoV Detection Kit. The control material was used to prepare an external positive control (20 µL) in pooled negative sputum matrix (180 µL) to the ratio of 1:10. The external control should be subjected to the entire test process including extraction.

8.4. Data Analysis

8.4.1 Interpretation of results

The test requires an NTC, and a prepared PC (see instructions above for preparing the positive control) to determine the validity of the experiment. In addition, it is recommended that external positive and negative controls are included for each test run and processed like clinical specimens, beginning with nucleic acid extraction. Each reaction includes PCRC in wells of samples as well as controls to check the validity of PCR. Interpretation criteria of the PCR results is in table 5.

Table 5. Interpretation of results (Controls)

Control	*Kaira* result (Ct value)			Interpretation
	RdRp (FAM)	E-gene (VIC/HEX)	PCRC** (Cy5)	
2019-nCoV Positive Control (Prepared)*	≤ 36	≤36	25 - 35	Positive Control valid
	> 36 or ND	> 36 or ND	25 - 35	Positive Control Invalid (Should retest if the expiration date of the kit is valid)
Negative Control (NTC)	ND	ND	25 - 35	Negative Control valid
	Detected	Detected	25 - 35	Negative Control Invalid (Should retest if the expiration date of the kit is valid)
External positive control *	ND	ND	25 - 35	RNA extraction control is invalid, repeat test run
	≤ 36	≤ 36	25 - 35	RNA extraction control is valid
External negative control	ND	ND	25 - 35	RNA extraction control is valid
	Detected	Detected	25 - 35	Negative Extraction Control Invalid, repeat test run

ND, Not detected, No amplification curve; Detected = Sigmoidal amplification curve observed

* Please refer to section *8.3.6 Application of external positive control.*

** PCRC must be detected for each control. If PCRC is not detected, the control is invalid.

Note. The interpretive criteria are the same for each thermocycler or instrument tested

Assessment of clinical specimen test results should be performed after the positive, negative and PCR controls have been examined and determined to be valid. If the controls are not valid, the patient results cannot be interpreted. Result criteria, result interpretation, and user actions are presented in tables 6 and 7. For the interpretation, the user will need to interpret the Ct values based on the algorithm in table 6 and 7.

Table 6. Criteria for result interpretation of specimen

	Ct value	Result
RdRp	≤ 37.5	Detected (+)
	>37.5 or ND	Not detected (-)
E-gene	≤36	Detected (+)
	> 36	Not detected (-)
PCRC	25 - 35	Valid

Table 7. Interpretation of results for clinical specimen*

RdRp (FAM)	E-gene (VIC/ HEX)	PCRC (Cy5)	Interpretation	Report	Actions
-	-	-	Invalid Result	INVALID	Repeat sample test. This re-test should include re-extraction steps. If an invalid in the re-test, report to sender as invalid.
-	-	+	SARS-CoV-2 Not Detected	NEGATIVE	Report results to sender.
-	+	+/-	Sarbecovirus Detected **	PRESUMPTIVE POSITIVE	Repeat sample test. If the same result is obtained, SARS-CoV-2 or other Sarbecovirus may be present (e.g., SARS-CoV-1).
+	+	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate public health authorities.
+	-	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate public health authorities.

Note: * A valid PCRC result is not required for a positive or presumptive positive specimen result.
** Sarbecovirus (SARS-CoV-1 orSARS-CoV-2) RNA is detected but SARS-CoV-2 specific RdRp target is not detected. Repeat testing. For samples with the same result on a repeated test, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.
Missing amplification of the SARS-CoV-2 specific target may be due to:
1) a sample at concentrations near or below the limit of detection of the test,
2) a mutation in the corresponding target region, or
3) other factors.

9. TROUBLESHOOTING

Comments and suggestions

PCR Control (PCRC) invalid results	
If the Cy 5 (PCRC) fluorescence signal was not detected in all wells (including controls).	• Extraction and/or PCR configuration error ☞ Follow the manufacture's protocol for RNA extraction. Repeat the assay, if necessary. See ***User's Guide*** **8. PROTOCOL** • Incorrect extraction or use of PCR kit ☞ Make sure that you use proper kits for the intended tests. • The kit may have spoiled due to bad storage or expiration. ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE**
If the Cy 5 (PCRC) fluorescence signal was not detected in particular wells.	• Inhibition of PCR ☞ Clinical samples may contain a variety of PCR inhibitors. Repeat the assay, including extraction. If PCRC still not detected, request collection of new specimen.

Positive Control (PC) invalid results	
If the FAM/HEX (PC) fluorescence signal was undetermined.	• The kit may have spoiled, due to bad storage or expiration. ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents if necessary. To repeat the test, please refer to the section 8.3.5 Quality Control/Assay Controls to prepare fresh positive control and run PCR. See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE** • Re-use of reagents ☞ Make sure not to re-use reagents. Re-use or repeated freeze/thaw cycles of reagents may affect the quality and the results of the assay. Repeat the assay with new reagents, if necessary. See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE and 7. General Precautions** • PCR Protocol error ☞ Review your reaction preparation procedure. Confirm the amount of PC used in a single well. See ***User's Guide*** **8.3.3 PCR Preparation** • Pipetting error. ☞ Review the pipetting technique and calibration.
No Template Control (NTC) invalid results	
If the FAM/HEX (PC) fluorescence signal was detected in NTC well.	• Contamination may have occurred. ☞ Make sure that workspace and instruments are decontaminated and repeat the assay. • The kit may have spoiled, due to bad storage or expiration. ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE** • PCR Protocol error ☞ Review your reaction preparation procedure. Confirm whether controls and samples are loaded in proper wells which are assigned through S/W protocol (especially NTC well(s)). See ***User's Guide*** **8.3.3 PCR Preparation** • There may have been a pipetting error. ☞ Review the pipetting technique and calibration.

9.1. Limitations

- This test is for *in vitro* diagnostic use under FDA Emergency Use Authorization. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- The *Kaira* 2019-nCoV Detection Kit does not include an internal control for RNA extraction/recovery or reverse transcription. A known SARS-CoV-2 positive sample or sample containing SARS-CoV-2 RNA (*in vitro* transcript or pseudovirus) should be tested with every batch of patient specimen to monitor the integrity of these process steps.
- A known SARS-CoV-2-positive sample or sample containing SARS-CoV-2 RNA (*in vitro transcript* or pseudovirus) should be extracted and amplified with each batch of patient specimens; additional controls should be tested in accordance laboratory practices and accreditation requirements.
- The performance of the Kaira 2019-nCoV Detection Kit was established using oropharyngeal swab and sputum specimens. Bronchoalveolar lavage, other swabs (nasopharyngeal, mid-turbinate and anterior nasal), nasopharyngeal/wash/aspirate and nasal aspirate are also considered acceptable specimen types for use with the Kaira 2019-nCoV Detection Kit but performance has not been established.
- Based on the *in silico* analysis, SARS-CoV-1 (Human SARS) and other Sarbecovirus may cross-react with the Kaira 2019-nCoV Detection Kit. SARS-CoV is not known to be currently circulating in the human population, therefore is highly unlikely to be present in patient specimens.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- PCR product contamination might occur in the laboratory, reagent preparation and cross-contamination of samples and will produce false positive results. The components of the test kit may decline due to improper transportation, storage or inaccurate preparation and will produce false negative results. False negative results may also arise from improper sample collection, degradation of the viral RNA during shipping/storage, using unauthorized extraction or assay reagents, mutation in the SARS-CoV-2 virus and failure to follow instructions for use
- Low viral load and excessive degradation in the samples may cause negative results. Thus, a negative result cannot completely exclude the presence of SARS-CoV-2 in the sample and should not be the sole basis of a patient management decision. Follow up testing should be performed according to the current CDC recommendations.
- Results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors.

[Conditions for Authorization for the Laboratory]

The Kaira 2019-nCoV Detection Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and 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 Kaira 2019-nCoV Detection Kit, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the Kaira 2019-nCoV Detection Kit will include with result reports of the test, 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 the Kaira 2019-nCoV Detection Kit will perform the test as outlined in the Instructions for Use. 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 the Kaira 2019-nCoV Detection Kit are not permitted.

C. Authorized laboratories that receive the Kaira 2019-nCoV Detection Kit will notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the Kaira 2019-nCoV Detection Kit 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 the Kaira 2019-nCoV Detection Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and OPTOLANE Technologies Inc. (via email: info@optolane.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

F. All laboratory personnel using the Kaira 2019-nCoV Detection Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the Kaira 2019-nCoV Detection Kit in accordance with the authorized labeling.

G. OPTOLANE Technologies, Inc., authorized distributors, and authorized laboratories using the Kaira 2019-nCoV Detection Kit will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] For ease of reference, this 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."

10. Performance Analysis

10.1. Analytical sensitivity (Limit of Detection (LoD))

10.1.1 Protocol

LoD studies were conducted using contrived samples comprised of pooled negative clinical sputum matrix and quantified full-length SARS-CoV-2 genomic RNA. Final SAR-CoV-2 concentrations ranged from $1.0x10^7$ copies/mL to $1.0x10^2$ copies/mL. Each sample replicate contained 200 µl and was independently extracted. The SARS-CoV-2 genomic RNA was spiked into the reagent (Carrier RNA+AVE buffer) at the lysis step of extraction procedure. Nucleic acid (RNA) and was extracted using the automated extraction instrument, QIAsymphony SP (Qiagen, Cat. 9001297) with its add-on kit, QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Cat. 937036). The final elution volume was 60 µL.

The final LoD of the assay was confirmed by testing 24 sample replicates of 2-fold diluted samples with each replicate prepared in a clinical sputum matrix and independently extracted. Testing was performed using three different instruments (ABI QS5, ABI 7500, and CFX96). The concentrations evaluated for the E gene and RdRp gene targets were 10,000, 5,000 and 2,500 copies/mL.

For all three instrument systems, the LoD of the assay was confirmed as 5,000 copies/mL i.e., when both the E gene and RdRp gene targets are detected for ≥95% of replicates tested. The LoD for each individual SARS-CoV-2 target was confirmed as 2,500 copies/mL for the E gene target and 5000 copies/mL for RdRp gene target. The results are summarized in table 8.

Table 8. Summary of LOD testing for E gene and RdRp gene by instruments (ABI QS5, 7500, and CFX96)

Instrument		ABI 7500			ABI QS5			Bio-Rad CFX96		
RNA (copies/mL)		10,000	5,000	2,500	10,000	5,000	2,500	10,000	5,000	2,500
E	Positive/Test	24/24	24/24	23/24	24/24	24/24	23/24	24/24	24/24	23/24
	Rate (%)	100.0	100.0	**95.8**	100.0	100.0	**95.8**	100.0	100.0	**95.8**
	Ave. Ct	33.95	34.90	35.78	33.95	34.95	35.72	33.77	34.72	35.73
	SD	0.33	0.43	0.21	0.39	0.43	0.24	0.19	0.31	0.24
RdRp	Positive/Test	24/24	23/24	16/24	24/24	23/24	13/24	24/24	23/24	14/24
	Rate (%)	100.0	**95.8**	66.7	100.0	**95.8**	54.2	100.0	**95.8**	58.3
	Ave. Ct	35.71	36.53	37.37	35.49	36.81	37.40	35.30	36.18	37.30
	SD	0.35	0.28	0.16	0.60	0.28	0.10	0.34	0.35	0.23
PCRC	Positive/Test	24/24	24/24	24/24	24/24	24/24	24/24	24/24	24/24	24/24
	Rate (%)	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Ave. Ct	28.79	29.08	29.28	29.35	29.51	29.57	30.59	30.53	30.65
	SD	0.17	0.18	0.30	0.24	0.17	0.16	0.27	0.30	0.29

10.2. Inclusivity/Reactivity

10.2.1. Inclusivity (Analytical sensitivity)

To ensure the performance of the test design, an *in silico* analysis was performed for the test inclusivity (Table 9). A multisequence alignment was generated with complete SARS-CoV-2 sequences of the US population in the GISAID database registered as of August 28, 2020 (n = 19,585). The amplicon region of interest was then compared for identity to the respective regions in the alignment. Homology of 99.9% for E-gene and 99.7% for RdRP gene was demonstrated between the target sequence and database genomes. The sequences that did not match fell into two categories. Less than 1% of the target sequences (E-gene (0.15%) and RdRP gene (0.78%)) showed one mismatch within the amplicon region. The mismatched regions were not located at the critical positions of the primers and probes and is not expected to affect test performance. Of the remaining unmatched sequences, less than 0.01%, showed mismatched in more than 2 nucleotides. Based on the high percentage of analyzed sequences having no mismatches, and of the very few sequences with mismatches, the likelihood of a false negative is expected to be very low.

Table 9. *In silico* Inclusivity Analysis*

Characteristic	E gene region	RdRP-gene
Total Length (nt)	113	100
Total # of Strains Evaluated	19,585	19,585
100% Match	19,569	19,528
1 Mismatch	31	152

2 Mismatch	2	0
>2 Mismatch	9	1

Target	Primer & Probe	No. of SARS-CoV-2 strains detection	Predicted Detection (Percent of Database Entries)
RdRP	CoV F	19,528/19,585	99.7%
	CoV R	19,535/19,585	99.7%
	CoV P	19,539/19,585	99.8%
E	CoV E F	19,575/19,585	99.9%
	CoV E R	19,569/19,585	99.9%
	CoV E P	19,569/19,585	99.9%

* The data was analyzed by using GISAID DB and reflected the latest data as of 08.28.2020

10.2.2. Cross-Reactivity (Analytical specificity)

Cross-reactivity was assessed using 11 microorganisms and viruses with the concentrations indicated. As shown in table 10, No cross-reactivity was observed for the 11 microorganisms and viruses evaluated (including 4 respiratory viruses (Corona 229E, Inf A H1N1, Inf A H3N2, Inf B)).

Table 10. Microorganisms and Viruses Evaluated for Cross-reactivity

Sample Name	Conc. (Copies/ µL)	QS 5			CFX 96			ABI 7500		
		RdRp	E	PCRC	RdRp	E	PCRC	RdRp	E	PCRC
Coronavirus 229E (KBPV-VR-9)	4.44 x10^5 copies/µL	ND	ND	28.57	ND	ND	28.41	ND	ND	28.53
		ND	ND	28.66	ND	ND	28.69	ND	ND	28.16
		ND	ND	29.15	ND	ND	27.46	ND	ND	27.95
Influenza virus A H1N1 (KBPV-VR-33D)	1.54 x 10^5 copies/µL	ND	ND	28.70	ND	ND	27.52	ND	ND	27.46
		ND	ND	28.75	ND	ND	27.81	ND	ND	28.55
		ND	ND	29.13	ND	ND	28.37	ND	ND	27.59
Influenza virus A H3N2 (KBPV-VR-71D)	4.4 x 10^5 copies/µL	ND	ND	29.48	ND	ND	27.95	ND	ND	28.27
		ND	ND	28.67	ND	ND	28.66	ND	ND	28.59
		ND	ND	28.35	ND	ND	28.47	ND	ND	28.57
Influenza virus B (KBPV-VR-72D)	1.03 x 10^5 copies/µL	ND	ND	28.36	ND	ND	28.37	ND	ND	27.18
		ND	ND	29.51	ND	ND	27.96	ND	ND	27.27
		ND	ND	29.11	ND	ND	27.48	ND	ND	27.21
Escherichia coli (EAEC) (MBC121)	13,000 copies/µL	ND	ND	28.01	ND	ND	27.52	ND	ND	28.66
		ND	ND	28.08	ND	ND	28.66	ND	ND	28.24
		ND	ND	28.52	ND	ND	28.71	ND	ND	28.95
Eastern Equine Encephalitis (MBC097)	16,000 copies/µL	ND	ND	28.34	ND	ND	27.41	ND	ND	27.65
		ND	ND	29.62	ND	ND	27.59	ND	ND	27.65
		ND	ND	28.96	ND	ND	27.24	ND	ND	27.59
Klebsiella pneumoniae (NDM-1) (MBC107)	12,000 copies/µL	ND	ND	28.46	ND	ND	27.26	ND	ND	28.19
		ND	ND	28.56	ND	ND	28.24	ND	ND	28.68
		ND	ND	28.64	ND	ND	28.55	ND	ND	28.95
Orientia tsutsugamushi (MBC106)	14,000 copies/µL	ND	ND	28.56	ND	ND	27.21	ND	ND	28.55
		ND	ND	28.69	ND	ND	28.34	ND	ND	27.20
		ND	ND	28.02	ND	ND	27.42	ND	ND	27.47
St. Louis Encephalitis Virus (MBC101)	12,500 copies/µL	ND	ND	29.56	ND	ND	28.24	ND	ND	28.22
		ND	ND	28.46	ND	ND	27.31	ND	ND	29.05
		ND	ND	28.02	ND	ND	27.34	ND	ND	27.27
Trypanosoma rangeii (MBC093)	11,000 copies/µL	ND	ND	28.66	ND	ND	27.33	ND	ND	27.20
		ND	ND	28.45	ND	ND	27.42	ND	ND	28.65
		ND	ND	28.67	ND	ND	27.81	ND	ND	27.68

Western Equine Encephalitis (MBC098)	15,000 copies/µL	ND	ND	29.66	ND	ND	28.20	ND	ND	27.95
		ND	ND	28.30	ND	ND	27.41	ND	ND	28.70
		ND	ND	28.18	ND	ND	28.32	ND	ND	29.57

In addition, *in silico* analysis was performed for 27 microbial pathogens and viruses including respiratory viruses. Blast homology search with primers and probes of the *Kaira* 2019-nCoV Detection Kit showed no homology for the organisms listed in table 11, with the exception of SARS-CoV-1 (Human SARS), for which the E gene primers and probe showed 100% homology.

Table 11. *In silico* analysis of cross-reactivity*

Pathogen	Strain #	GenBank Acc #	% Homology								
			RdRp			E			PCRC		
			Primer (F)	Primer (R)	Probe	Primer (F)	Primer (R)	Probe	Primer (F)	Primer (R)	Probe
Human coronavirus HKU1	75	All	0	0	0	0	0	0	0	0	0
Human coronavirus NL63	147	All	0	0	0	0	0	0	0	0	0
Human coronavirus OC43	214	All	0	0	0	0	0	0	0	0	0
Human coronavirus 229E	25	All	0	0	0	0	0	0	0	0	0
SARS-CoV-1**	50	All	0	0	0	100	100	100	0	0	0
MERS-coronavirus	303	All	0	0	0	0	0	0	0	0	0
Human Adenovirus	390	All	0	0	0	0	0	0	0	0	0
Human Metapneumovirus	162	All	0	0	0	0	0	0	0	0	0
Parainfluenza virus 1	27	All	0	0	0	0	0	0	0	0	0
Parainfluenza virus 2	58	All	0	0	0	0	0	0	0	0	0
Parainfluenza virus 3	88	All	0	0	0	0	0	0	0	0	0
Parainfluenza virus 4	14	All	0	0	0	0	0	0	0	0	0
Enterovirus D68	225	All	0	0	0	0	0	0	0	0	0
Respiratory syncytial virus	228	All	0	0	0	0	0	0	0	0	0
Rhinovirus	21	All	0	0	0	0	0	0	0	0	0
Chlamydia pneumoniae	13	All	0	0	0	0	0	0	0	0	0
Haemophilus influenzae	34	All	0	0	0	0	0	0	0	0	0
Legionella pneumophila	19	All	0	0	0	0	0	0	0	0	0
Mycobacterium tuberculosis	38	All	0	0	0	0	0	0	0	0	0
Streptococcus pneumoniae	29	All	0	0	0	0	0	0	0	0	0
Streptococcus pyogenes	137	All	0	0	0	0	0	0	0	0	0
Bordetella pertussis	170	All	0	0	0	0	0	0	0	0	0
Mycoplasma pneumoniae	140	All	0	0	0	0	0	0	0	0	0
Candida albicans	5	All	0	0	0	0	0	0	0	0	0

Pseudomonas aeruginosa	56	All	0	0	0	0	0	0	0	0	0
Staphylococcus epidermidis	41	All	0	0	0	0	0	0	0	0	0
Streptococcus salivarius	26	All	0	0	0	0	0	0	0	0	0

* Note: The analysis was performed using ThermoBLAST from dnasoftware (DNASOFTWARE™, USA)
** SARS-CoV-1 will generate a positive result for the E gene target.

10.3. Clinical Evaluation

To evaluate the clinical performance of *Kaira* 2019-nCoV Detection Kit, a clinical investigation with clinical specimens collected from patients who are suspected for COVID-19 was performed. The clinical evaluation was designed for randomized, single-blinded comparative clinical evaluation. Nucleic acid (RNA) was extracted by using the automated extraction instrument, QIAsymphony SP with its reagents, QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Cat. 937036). Clinical specimens were divided into two aliquots. One was extracted for *Kaira* 2019-nCoV detection kit and the other for a FDA-authorized comparator real-time RT-PCR assay. Clinical specimens (oropharyngeal swabs and sputum) taken from COVID-19 suspected patients were extracted within 48hrs of sampling. Prior to testing with the Kaira 2019-nCoV Detection Kit, extraction was performed using QIAsymphony SP (Qiagen, Cat. 9001297) instrument with the QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Cat. 937036). For PCR, the Bio-Rad CFX96 system was used.

For the comparator assay, specimens were separately extracted according to the instructions for use. The extracted nucleic acid specimens were stored frozen prior to performing real-time RT-PCR with the Kaira 2019-nCoV Detection Kit.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined through a comparative investigation of results from the Kaira 2019-nCoV Detection Kit and the comparator assay. Table 12 includes the numbers of positive and negative oropharyngeal swab and sputum specimens included in the study based on results from the comparator method.

Table 12. Specimen types used in the clinical performance evaluation (Unit: Case)

Oropharyngeal swabs	
Negative	16
Positive	15

Sputum	
Negative	14
Positive	15

The results of 30 positive and 30 negative samples tested below showed that, for OP, the Positive Percent Agreement (PPA) is 100% (95% CI, 79.62 ~ 100%) and Negative Percent Agreement (NPA) is 100.0% (95% CI, 80.64 ~ 100%). For sputum, PPA is 100.0% (95% CI, 79.62 ~ 100%) and NPA is 100% (95% CI, 78.47 ~ 100%). The *Kaira* 2019-nCoV Detection Kit did not show any false positives or false negatives for the detection of SARS-CoV-2 in comparison with the authorized comparator as shown in Table 13. Table 14 includes result details for individual specimens for both the *Kaira* 2019-nCoV Detection Kit and the comparator.

Table 13. Clinical performance summary of *Kaira* 2019-nCoV Detection Kit vs. comparator test

Oropharyngeal swab		**EUA Authorized real-time RT-PCR comparator assay**		**Total**
		Positive	**Negative**	
Investigation reagents (Kaira 2019-nCoV Detection Kit)	**Positive**	15	0	15
	Negative	0	16	16
Total		15	16	31

Sputum	**EUA Authorized real-time RT-PCR comparator assay**		**Total**
	Positive	**Negative**	

Investigation reagents (Kaira 2019-nCoV Detection Kit)	Positive	15	0	15
	Negative	0	14	14
Total		15	14	29

Table 14. Clinical performance evaluation result of *Kaira* 2019-nCoV Detection Kit

[Performance table for Oropharyngeal Swab specimens]

Specimen #	Specimen Type	Kaira 2019-nCoV Detection Kit Results				Comparator Assay Results				
		E	RdRp	PCRC	Final	E	RdRp	N	IC	Final
DK01	P.S	23.38	25.03	29.67	Positive	22.2	22.64	23.54	30.57	Positive
DK03	P.S	34.49	36.71	29.71	Positive	34.55	34.45	34.86	22.16	Positive
DK05	P.S	28.15	33.06	29.67	Positive	27.17	29.83	29.43	28.66	Positive
DK07	P.S	33.52	35.51	29.36	Positive	31.81	33.09	33.65	23.05	Positive
DK09	P.S	24.84	26.43	29.25	Positive	22.8	23.52	23.9	31.22	Positive
DK11	P.S	N/A	N/A	30	Negative	N/A	N/A	N/A	22.26	Negative
DK13	P.S	N/A	N/A	29.82	Negative	N/A	N/A	N/A	22.02	Negative
DK15	P.S	N/A	N/A	29.99	Negative	N/A	N/A	N/A	21.91	Negative
DK17	P.S	N/A	N/A	29.94	Negative	N/A	N/A	N/A	22.34	Negative
DK19	P.S	N/A	N/A	30.03	Negative	N/A	N/A	N/A	21.94	Negative
DK21	P.S	33.29	35.61	29.9	Positive	31.12	32.16	32.44	22.91	Positive
DK23	P.S	23.35	25.16	29.73	Positive	22.69	23.07	23.52	30.52	Positive
DK25	P.S	29.53	31.56	30.01	Positive	28.34	28.55	29.02	28.41	Positive
DK27	P.S	21.93	24.3	29.84	Positive	21.2	21.82	22.23	32.55	Positive
DK29	P.S	20.18	22.29	33.82	Positive	20.34	21.03	21.61	31.38	Positive
DK31	P.S	N/A	N/A	29.97	Negative	N/A	N/A	N/A	22.81	Negative
DK33	P.S	N/A	N/A	30.02	Negative	N/A	N/A	N/A	21.99	Negative
DK35	P.S	N/A	N/A	29.95	Negative	N/A	N/A	N/A	22.2	Negative
DK37	P.S	N/A	N/A	30.17	Negative	N/A	N/A	N/A	22.61	Negative
DK39	P.S	N/A	N/A	30.09	Negative	N/A	N/A	N/A	22.14	Negative
DK41	P.S	25.67	30.17	29.84	Positive	25.9	25.47	25.81	29.63	Positive
DK43	P.S	33.06	35.36	29.9	Positive	32.62	33.39	33.46	22.76	Positive
DK45	P.S	27.15	33.03	28.95	Positive	26.64	26.92	27.01	28.47	Positive
DK47	P.S	18.16	22.29	29.18	Positive	17.64	18.09	18.73	33.51	Positive
DK49	P.S	24.89	30.4	28.9	Positive	23.91	24.35	24.82	29.74	Positive
DK51	P.S	N/A	N/A	28.92	Negative	N/A	N/A	N/A	22.04	Negative
DK52	P.S	N/A	N/A	29.01	Negative	N/A	N/A	N/A	21.72	Negative
DK54	P.S	N/A	N/A	28.94	Negative	N/A	N/A	N/A	22.37	Negative
DK56	P.S	N/A	N/A	28.98	Negative	N/A	N/A	N/A	22.68	Negative
DK58	P.S	N/A	N/A	29.08	Negative	N/A	N/A	N/A	21.85	Negative
DK60	P.S	N/A	N/A	29.21	Negative	N/A	N/A	N/A	22.46	Negative

[Performance table for Sputum specimens]

Specimen #	Specimen Type	Kaira 2019-nCoV Detection Kit Results				Comparator Assay Results				
		E	RdRp	PCRC	Final	E	RdRp	N	IC	Final
DK02	Sputum	26.35	27.27	29.63	Positive	25.16	25.28	26.13	28.94	Positive
DK04	Sputum	33.33	35.91	29.74	Positive	32.71	33.7	32.35	22.39	Positive
DK06	Sputum	31.7	33.22	30	Positive	30.05	30.51	30.86	24.34	Positive
DK08	Sputum	33.95	36.8	29.75	Positive	32.87	34.1	33.96	23.34	Positive
DK10	Sputum	31.1	33.17	29.53	Positive	28.86	30.26	30.83	26.45	Positive
DK12	Sputum	N/A	N/A	29.92	Negative	N/A	N/A	N/A	21.99	Negative
DK14	Sputum	N/A	N/A	30.04	Negative	N/A	N/A	N/A	22.5	Negative
DK16	Sputum	N/A	N/A	29.95	Negative	N/A	N/A	N/A	22.81	Negative
DK18	Sputum	N/A	N/A	30.01	Negative	N/A	N/A	N/A	21.86	Negative
DK20	Sputum	N/A	N/A	29.91	Negative	N/A	N/A	N/A	22.67	Negative
DK22	Sputum	22.77	24.69	29.53	Positive	23.04	23.41	23.86	31.04	Positive
DK24	Sputum	22.38	24.49	29.49	Positive	21.84	22.4	22.31	31.83	Positive
DK26	Sputum	18.49	20.36	31.21	Positive	18.2	18.41	18.69	39.61	Positive
DK28	Sputum	19.86	21.65	30.33	Positive	19.16	19.6	19.87	32.29	Positive
DK30	Sputum	28.04	30.63	30.04	Positive	28.25	29.08	29.68	28.35	Positive
DK32	Sputum	N/A	N/A	30.06	Negative	N/A	N/A	N/A	22.15	Negative
DK34	Sputum	N/A	N/A	30.01	Negative	N/A	N/A	N/A	21.72	Negative
DK36	Sputum	N/A	N/A	30.02	Negative	N/A	N/A	N/A	22.44	Negative
DK38	Sputum	N/A	N/A	30	Negative	N/A	N/A	N/A	21.89	Negative
DK40	Sputum	N/A	N/A	29.99	Negative	N/A	N/A	N/A	22.47	Negative
DK42	Sputum	31.67	33.44	29.87	Positive	31.87	31.56	31.43	22.81	Positive
DK44	Sputum	33.84	35.07	30.04	Positive	32.84	34.23	34.58	22.62	Positive
DK46	Sputum	21.13	25.92	27.74	Positive	20.55	21.08	21.36	30.77	Positive
DK48	Sputum	26.65	31.71	29.97	Positive	25.4	25.96	26.04	29.77	Positive
DK50	Sputum	35.52	36.94	29.89	Positive	32.04	33.64	33.49	22.93	Positive
DK53	Sputum	N/A	N/A	29.11	Negative	N/A	N/A	N/A	22.28	Negative
DK55	Sputum	N/A	N/A	28.94	Negative	N/A	N/A	N/A	21.94	Negative
DK57	Sputum	N/A	N/A	28.97	Negative	N/A	N/A	N/A	22.2	Negative
DK59	Sputum	N/A	N/A	29.1	Negative	N/A	N/A	N/A	22.55	Negative

10.4. 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 confirm the LoD. The RNA extraction was performed by using QIAsymphony SP (Qiagen, Cat. 9001297) instrument with its add-on kit, QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Cat. 937036). The Bio-Rad CFX96 instrument was used for amplification during the panel evaluation. The results are summarized in Table 15.

Table 15: 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	NP swab	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

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NCCLS. Quantitative Molecular Methods for Infectious Diseases; Approved Guideline. NCCLS document MM6-A.

NCCLS. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. NCCLS document EP17-A.

12. SYMBOLS

REF	Catalog number		Temperature limitation
IVD	*In vitro* diagnostic medical device	Σ	Contains sufficient for test
	Manufacturer		Caution, consult accompanying documents
LOT	Batch code		Expiration date
	Consult instructions for use	Rx Only	Prescription use only

OPTOLANE Technologies, Inc.
6F, 20, Pangyoyeok-ro 241beon-gil Bundang-gu Seongnam-si, Gyeonggi-do 13494 Republic of Korea
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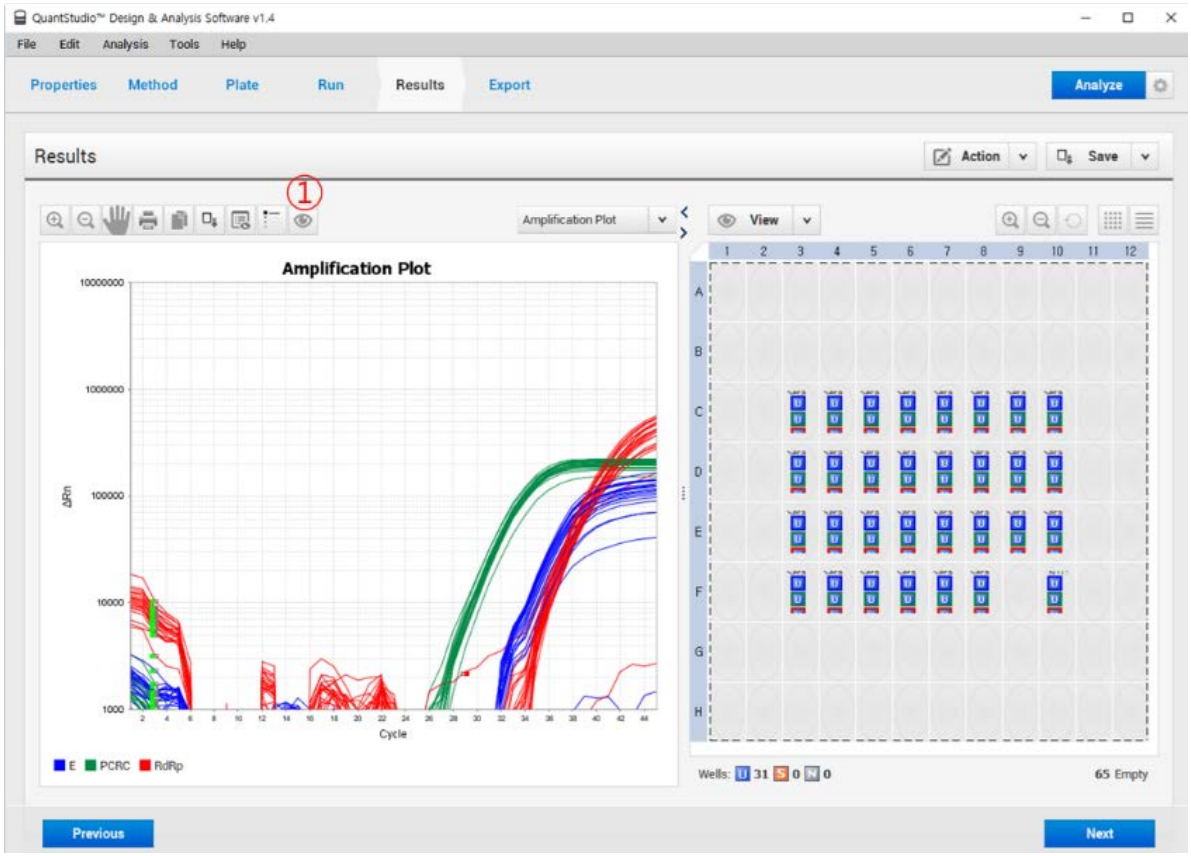
Email: info@optolane.com Website: www. optolane.com

13. APPENDIX

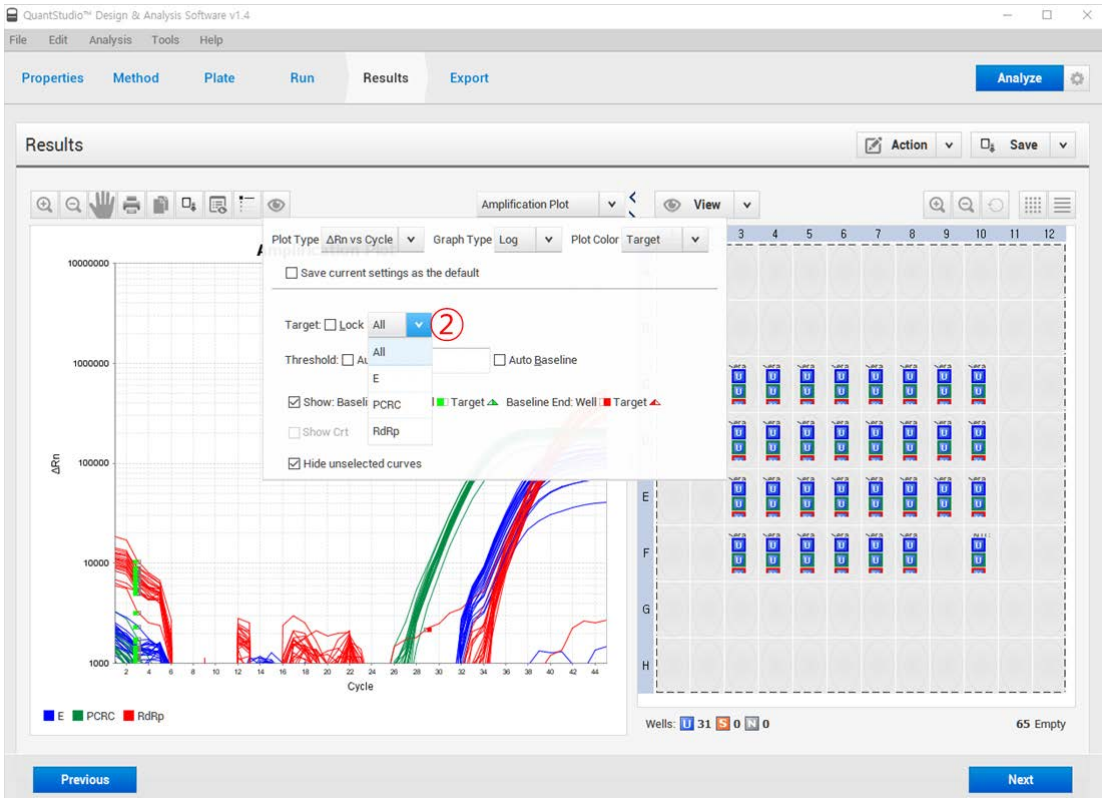
Manual selection of threshold values in qPCR instruments.

[Quant Studio 5]

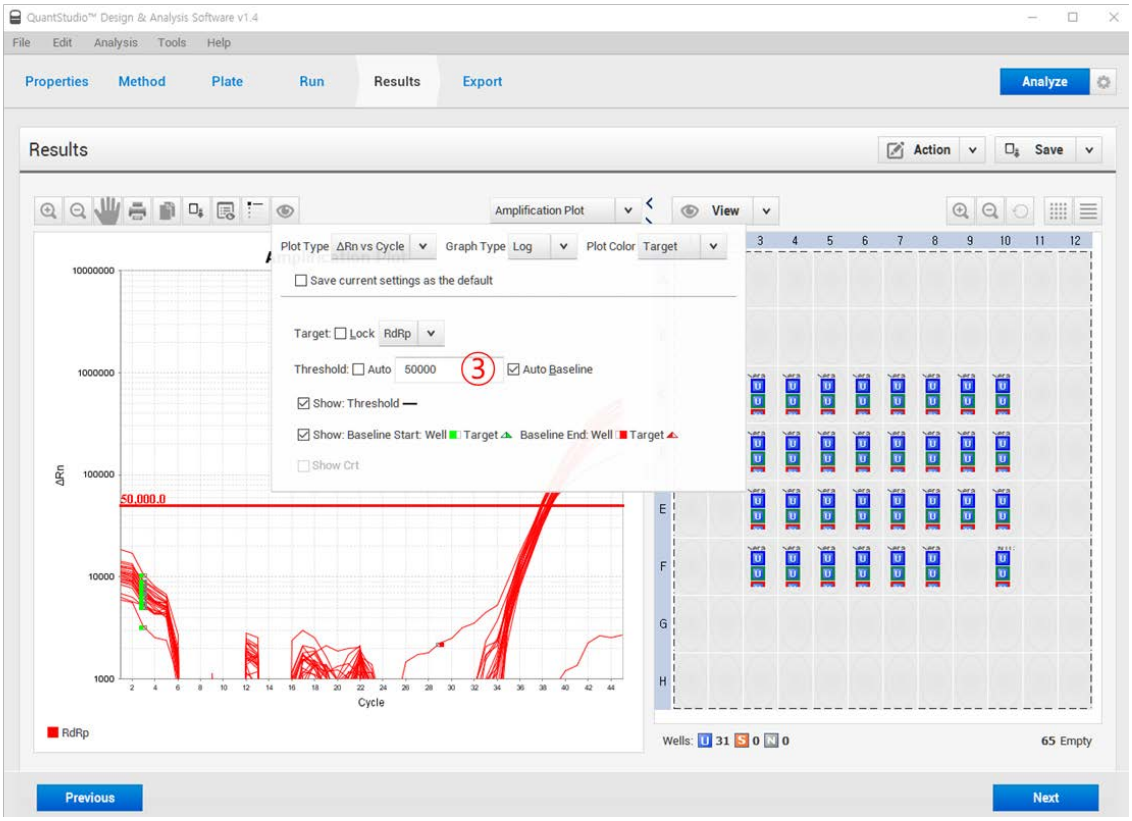
1. Press eye shaped icon ①



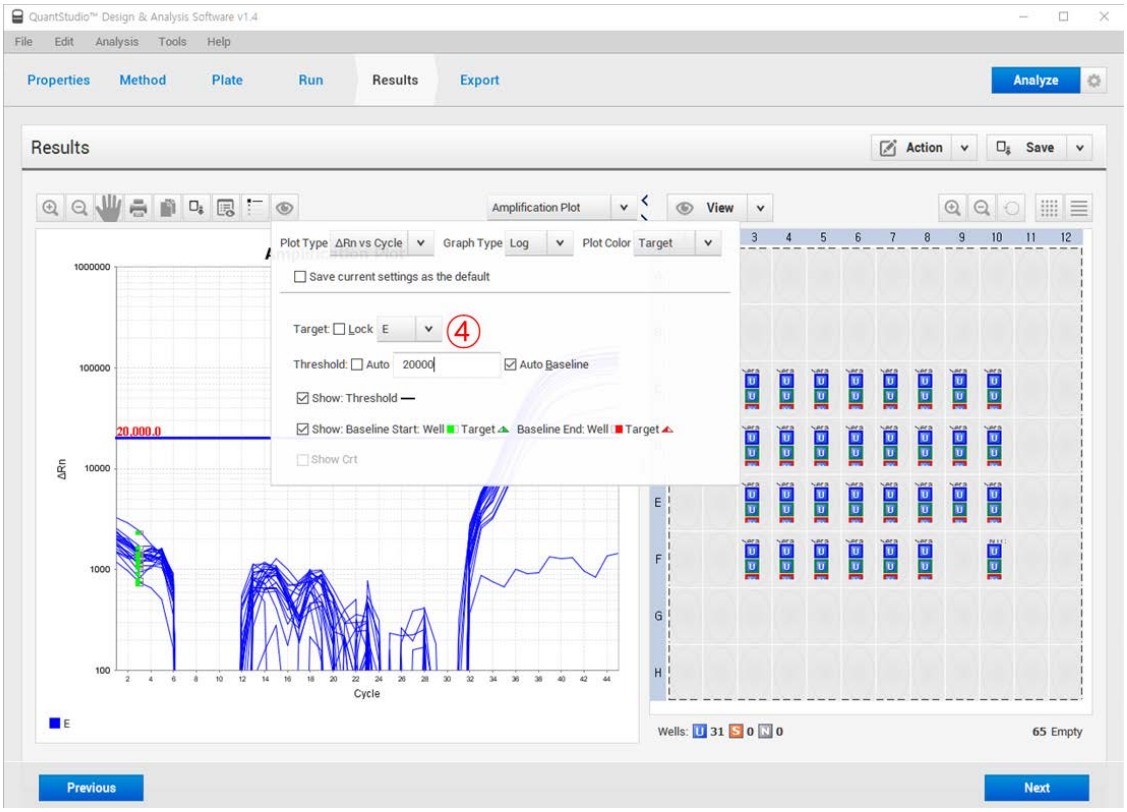
2. Select RdRp in the options of Target (②)



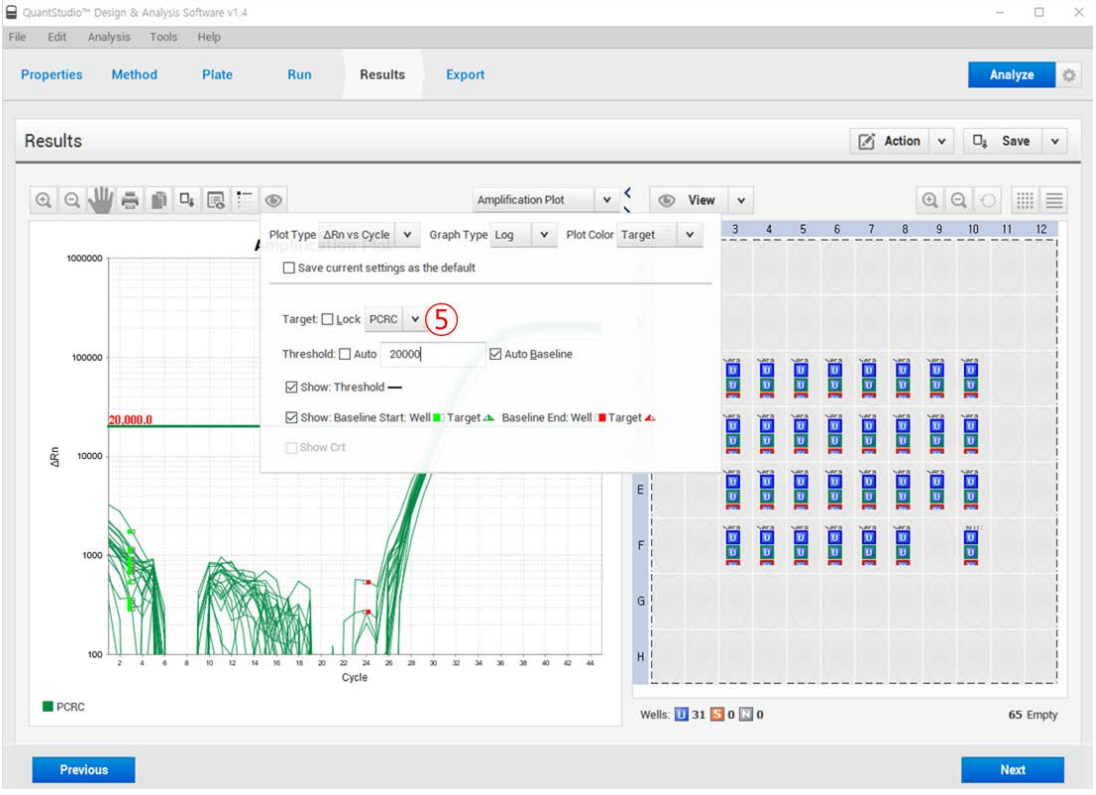
3. Type 50,000 on the blank of "Threshold".



4. Select "E" (④) and input 20,000 in the "Threshold"



5. Select "PCRC (⑤)" in the "Target" option and input 20,000 in the "Threshold"



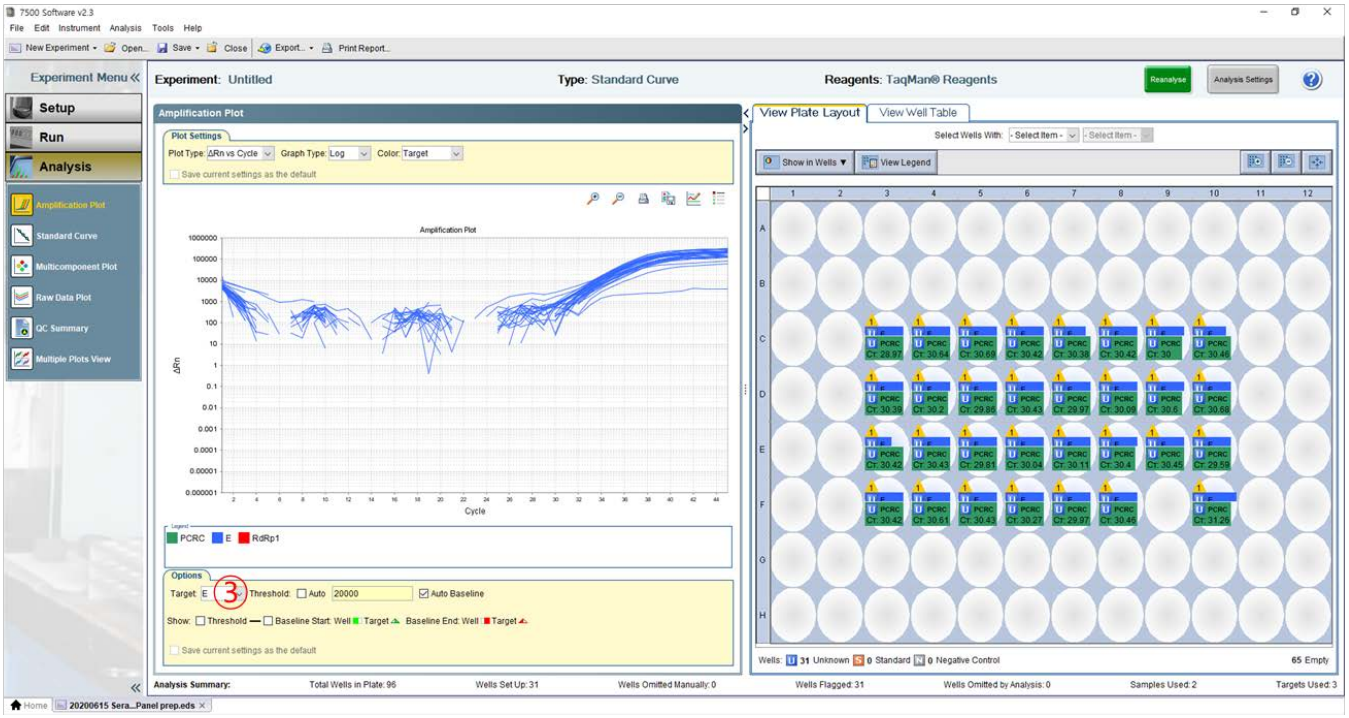
6. Press "Analyze (⑥)" to re-analyze the result with new input values.

[ABI 7500]

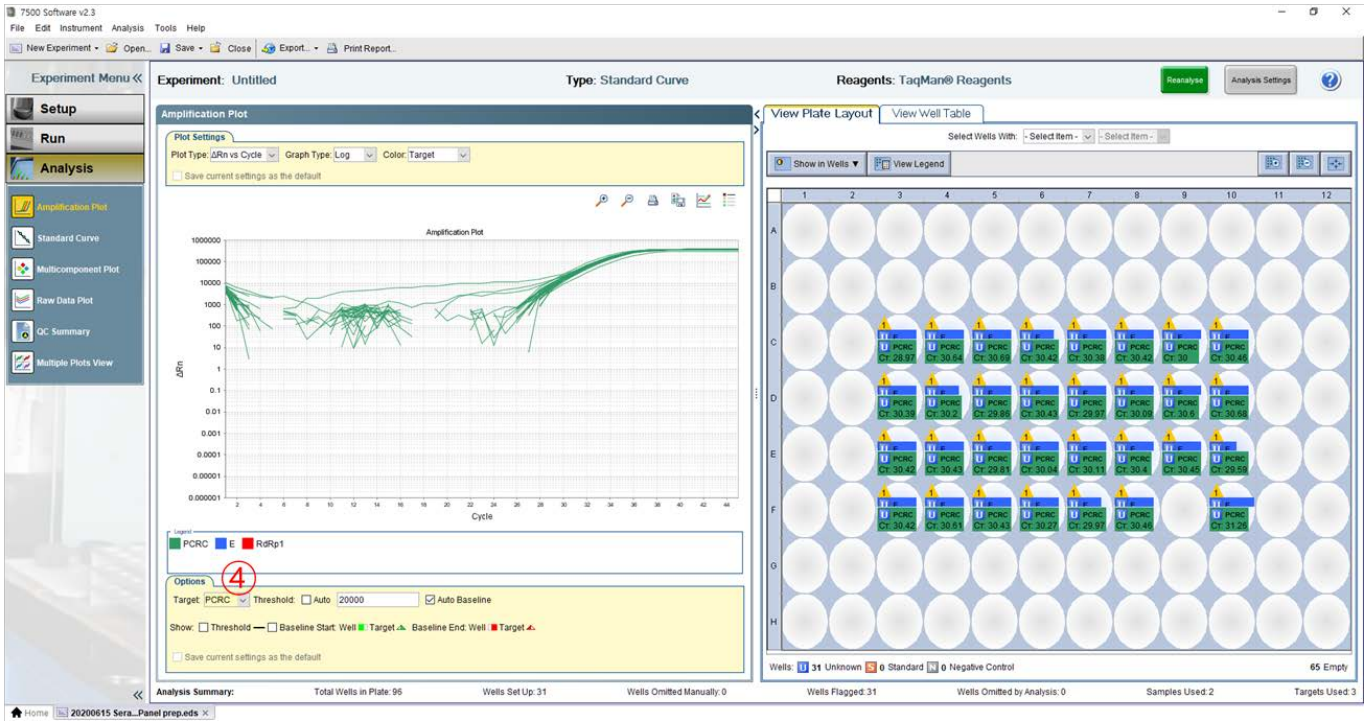
1. Press options in the "Target (①)" and select "RdRp"

2. Unselect "Auto" next to the "Threshold" and type 50,000 in the blank

3. Select E assay in the same way as in step 2 and enter the value of 20,000



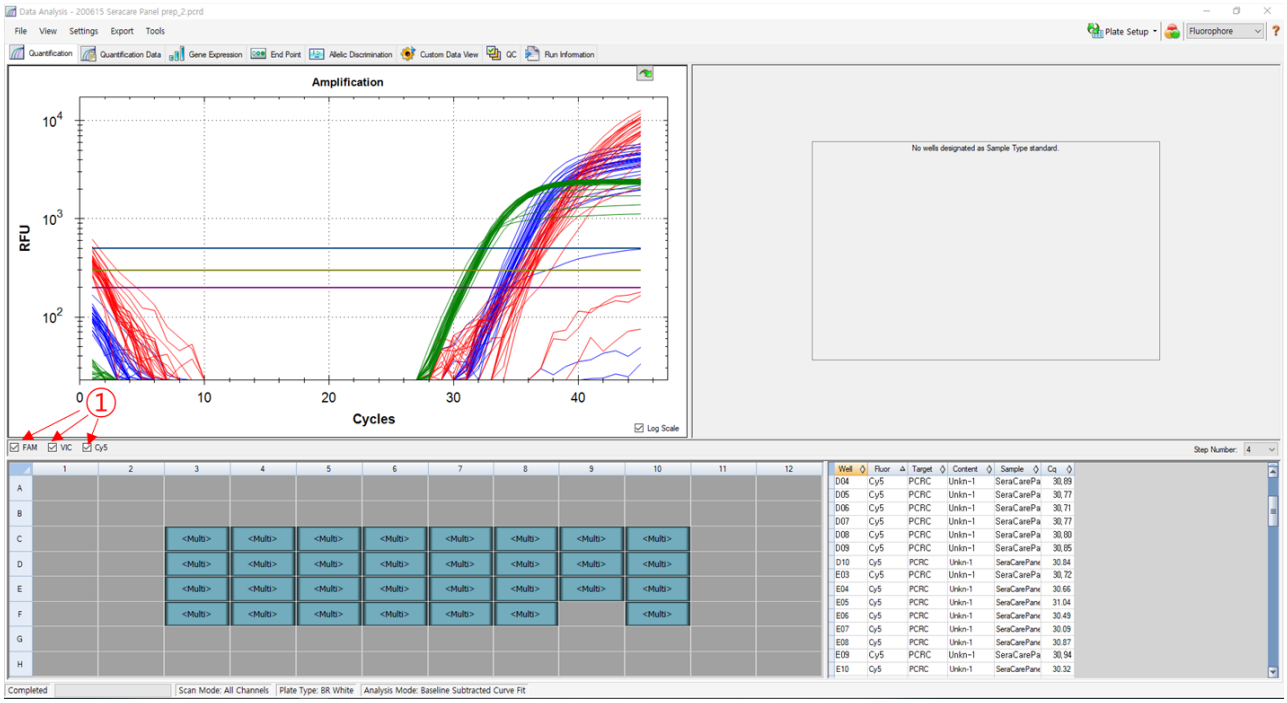
4. Select PCRC in the same way as before and enter the value of 20,000



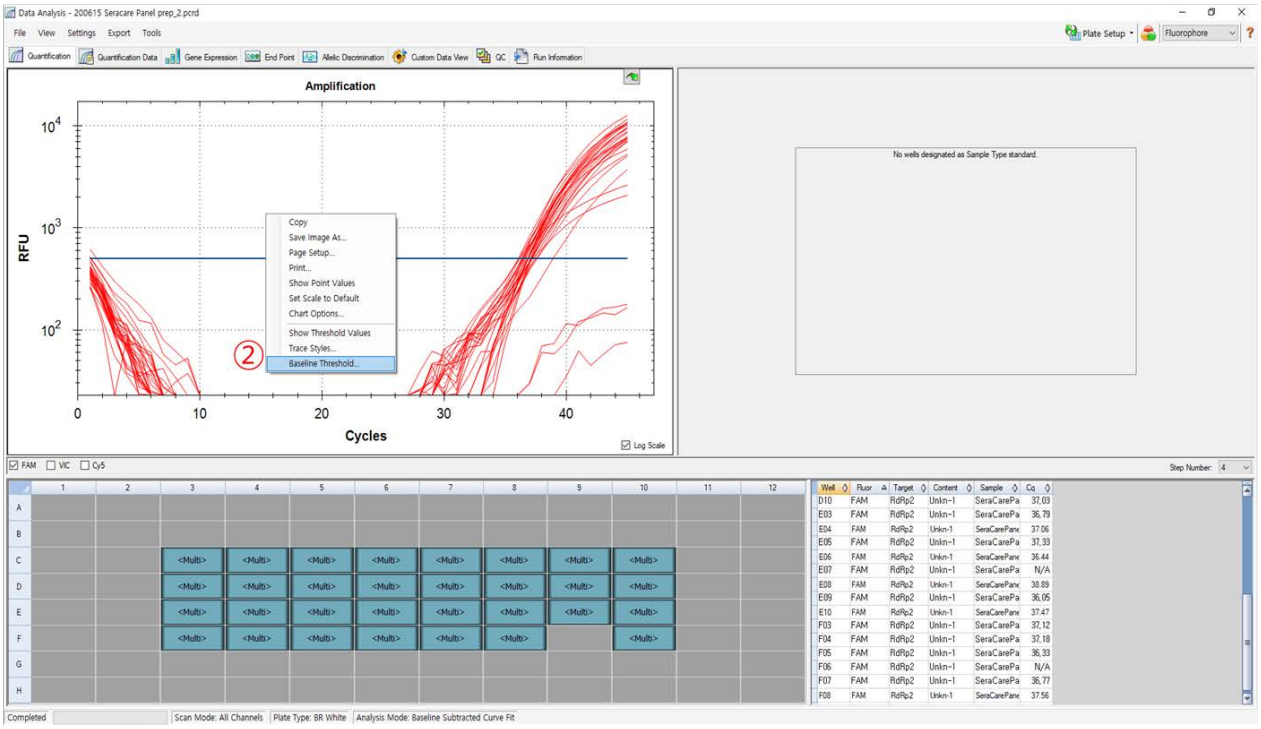
5. Press the Analyze button to reanalyze the newly entered threshold value

[BioRad CFX-96]

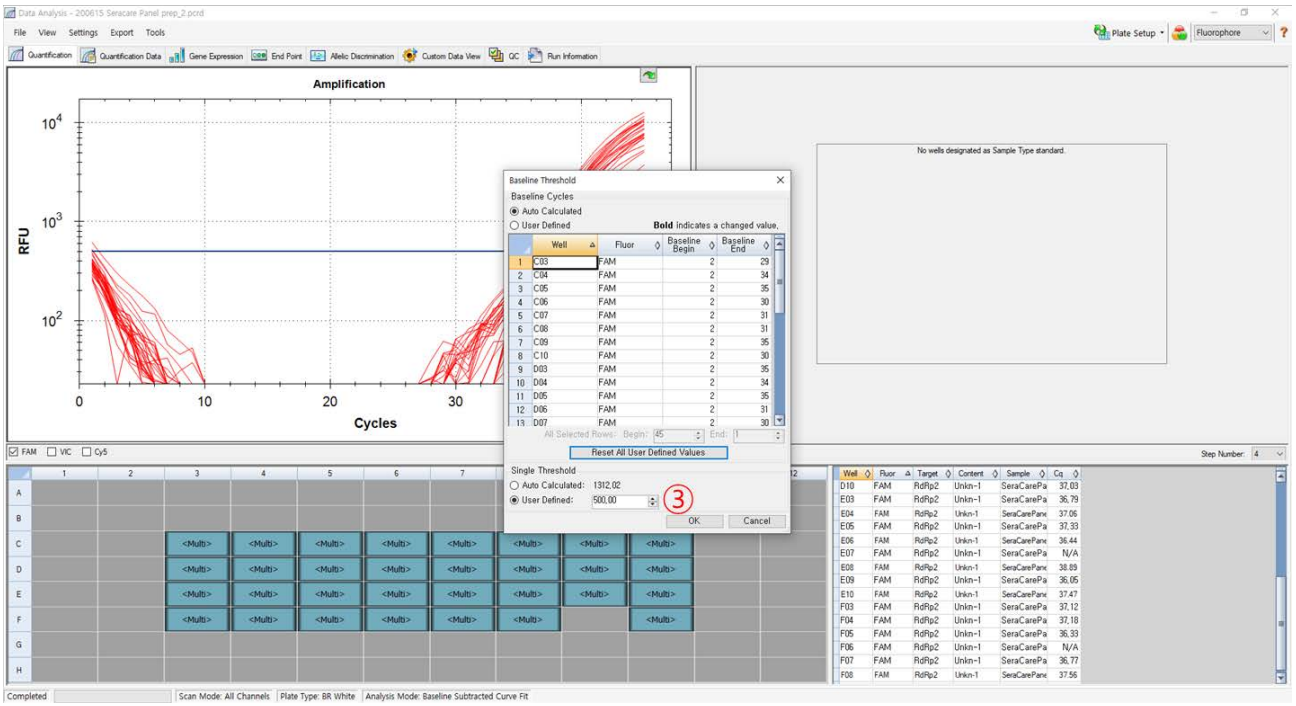
1. Deselect all fluorescence except FAM



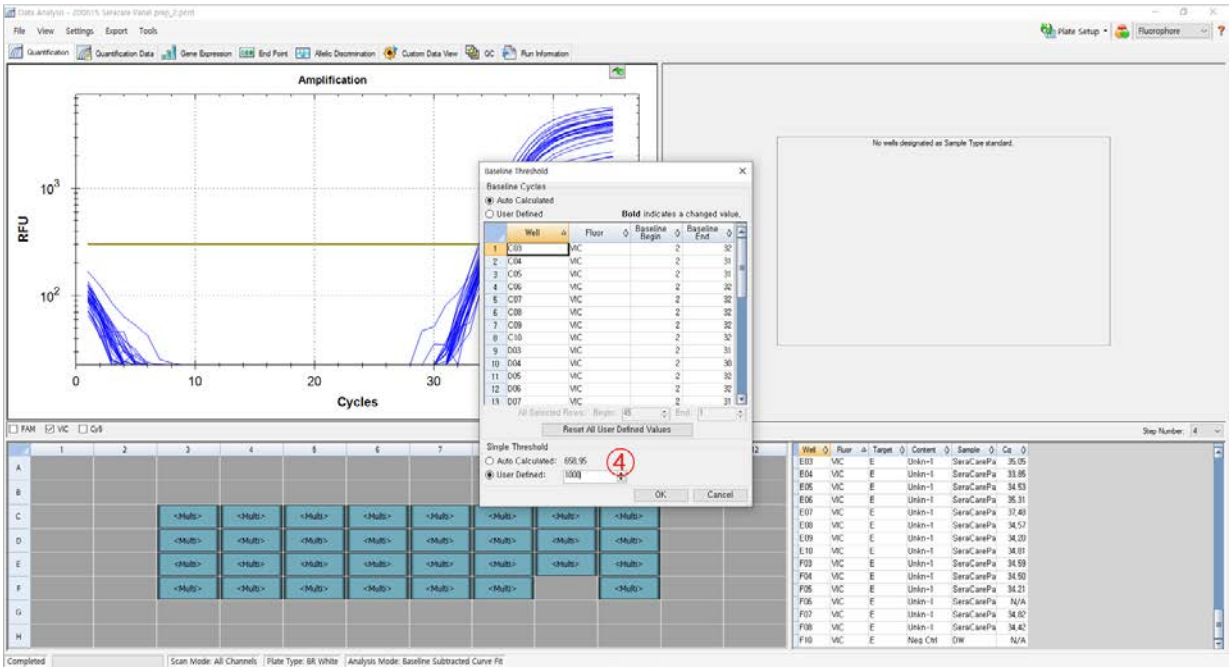
2. Right-click on the graph screen and select Baseline Threshold.



3. Under Single Threshold, select User Defined, enter 500 and click OK



4. After selecting VIC fluorescence in the same way as the FAM fluorescence setting, select User Defined at Single threshold, enter 1,000, and click OK.



5. Select Cy5 in the same way as FAM and VIC, enter 300 in the User Defined input window and click OK.