DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit

INSTRUCTIONS FOR USE (IFU)

Revision 2.10 | 11.2020

Rx only

For *in vitro* Diagnostic Use

For use under Emergency Use Authorization (EUA) only

SolGent Co., Ltd.
Solutions for Genetic Technologies

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Intended Use

The DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit is a real-time reverse transcriptase (RT)-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum 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, 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. The agent detected may not be the definite cause of disease. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive 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 DiaPlexQ™ Novel Coronavirus (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. The DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product description

1. Summary and Explanation

Respiratory infections caused by viruses appears mainly in children, the elderly and immunocompromised patients. The major respiratory infection viruses are known as Influenza virus, Parainfluenza virus (PIV), Respiratory syncytial virus (RSVs), Enterovirus, Adenovirus, etc. In recent years, respiratory infections of Rhinovirus, Coronavirus and Metapneumovirus (MPV) have been increasing, and Bocavirus has been included in the major respiratory virus tests.

Coronavirus is a virus that can infect animals and humans. There are six known coronaviruses that can infect humans. Four of them are known as viruses that cause diseases such as the common cold, and the other two are MERS-CoV (Middle East Respiratory Syndrome Coronavirus) and SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus), which have been fatal to humans.

SARS-COV-2, which originated in Wuhan, China in 2019, is transmissible between humans, with up to a 14-day incubation period, and has been reported to have a lower mortality rate and a higher incidence than SARS-CoV or MERS-CoV. Sequencing of the virus revealed that SARS-CoV-2 was 89.1% homologous to bat-derived SARS (severe acute respiratory syndrome)-like coronaviruses, bat-SL-CoVZC45, bat- SL-CoVZXC21), and 79% homologous to SARS-CoV. It is also very important to accurately diagnose COVID-19, since it has sequences similar to viruses of the same genus.

Respiratory virus testing requires the selection of appropriate test methods depending on the characteristics of the hospital's patient population and laboratory conditions. Antigen testing, virus culture, and molecular biological methods have been used to detect viruses. Among them, the molecular biological method using Reverse Transcription Polymerase Chain Reaction (RT-PCR) is analytically highly sensitive and is recognized as a standard method for detecting viruses that cannot be cultured or that exist in low concentrations. Recently, one-step RT-PCR, in which reverse transcription and polymerase chain reaction (PCR) amplification can be performed in one tube, allows for the accurate identification of many viruses. The DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit is a real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 nucleic acid extracted from nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum.

2. Principles of the Procedure

DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit is an in vitro diagnostic reagent for qualitative detection of ORF1a and the N gene of SARS-CoV-2 by processing through Multiplex OneStep qRT-PCR.

< Detection Target Information >

Target virus	Target genes
SARS-CoV-2	*N* gene
	ORF1a
	PCRC

The kit includes 2X OneStep qRT-PCR Buffer, OneStep qRT-PCR Enzyme mix (including reverse transcriptase, DNA polymerase and RNase inhibitor) and Primer & Probe Mixture. We also provide a DNA-based Control Template (2019-nCoV), to monitor PCR process and reagent integrity.

< Fluorescence Information >

Target genes	5′ Fluorophore	3′ Quencher
N gene	FAM	BHQ1
ORF1a	JOE / VIC	BHQ1
PCRC	Texas Red / Cal Fluor Red 610	BHQ2

The kit does not include a "Reference dye"

(E.g. Set up the reference dye to "None" in the ABI 7500 / 7500 Fast program)

(* ABI 7500 / 7500 Fast set in "JOE" and "Texas Red", Bio-Rad CFX96™ set in "VIC" and "Cal Fluor Red 610")

Precautions and handing requirements

- For in vitro diagnostic use.
- For emergency use.
- Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- The results should be interpreted in accordance with the result analysis section of the IFU after processing on a Compatible Real-Time PCR thermocycler.
- Only use PCR tubes that are compatible with the applicable PCR machine.
- Wear protective disposable powder-free gloves, a laboratory coat, and eye protection when handling specimens.
- Always wear protective disposable powder-free gloves when handling kit components in order to avoid any contamination that can affect the test result.
- Do not reuse disposable tips, gloves, test tubes etc.
- Be careful not to let the reagents in this test come into contact with skin, eyes or mucous membranes. If contact occurs, wash off immediately with plenty of water.
- The work area should be disinfected prior to and after use.
- All reagents should be stored by following the specified storage conditions before and after use.
- Do not leave the reagent cap open.
- Only use sterile pipette tips.
- Dispose of unused kit reagents and human specimens according to local, state and federal regulations. Do not smoke, drink, eat, handle contact lenses or apply make-up in areas where kit reagents and/or human specimens are being used. Follow universal precautions and treat all specimens, samples and used kit components as potentially infectious.
- Store Control Template separately in order to prevent contamination.
- Please thaw the product on ice.
- When using the product, do not mix components from different kit lots.
- If an item arrives broken or damaged during transport, contact SolGent Co., Ltd.

Product warranty and liability

- The product expiry date is 1 year after the manufacturing date.
- Only use the protocol described in this package insert. Deviations from the protocol may give erroneous results.
- Exchange is not possible in case of a problem due to the user's carelessness or fault.
- Do not repeat freeze-thaw over 5 times.

Safety warnings and first aid measures

- Avoid contact with eyes, skin and respiratory system.
- Eye contact: Wash eyes with lots of flowing water.
- Consult with physician in case of irritation.
- Skin contact: Wash affected skin area thoroughly with soap and water.

Precautions

- Do not use product after expiration date.
- Immediately use this kit after opening.
- Specimen quality and the integrity of the extracted nucleic acid may affect test results.
- False results may occur due to contamination.
- Dispose of unused reagents and waste in accordance with county, federal, provincial, state and local regulations.
- Dispose of used devices, pipette tips and specimen tubes according to your institution's safety guidelines for hazardous material.

Contents

Components	SQD52-K100
OneStep qRT-PCR Enzyme mix (2019-nCoV)	200 ㎕ x 1 ea
2X OneStep qRT-PCR Buffer (2019-nCoV)	1 ㎖ x 1 ea
Primer & Probe Mixture (2019-nCoV)	300 ㎕ x 1 ea
Control Template (2019-nCoV)	100 ㎕ x 1 ea
RNase free Water	1 ㎖ x 1 ea

Storage and Handling

- **DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit** should be stored at -20℃ ± 5℃ and kept away from sunlight. All components should be stored under recommended storage conditions.

Model Name	Storage	Period of use
DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit	-20℃ ± 5℃	1 year

- The expiry date of each component of the product is 1 year from the date of manufacture.
- Do not use product beyond the expiration date.
- Please thaw the product on the ice.
- Do not freeze-thaw more than 5 times.
- If there are or have been transportation problems, or the protective packaging is damaged, do not use the kit and contact your distributor for guidance.

Material to be supplied by User

- Micro-centrifuge tube
- Micro-centrifuge
- Vortexer
- Pipettes/ pipette filter tips
- Laboratory freezers
- Disposable latex
- Cooling device or ice
- Tubes, plates, and other consumables
- QIAGEN QIAamp Viral RNA Mini Kit (Cat. # 52904 or #52906) or the MagNA Pure 96 nucleic acid extraction system with software V3.1 and the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Cat. #06 543 588 001) with External Lysis Buffer (Cat. #06 374 913 001)
- Real-Time PCR Instrument System and data analysis software
- AccuPlex™ SARS-CoV-2 Reference Material Kit (Cat. No. 0505-0126)

Compatible Real-Time PCR thermocycler

- Applied Biosystems™ 7500 Real-Time PCR Instrument System with software V2.0.6
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument System with software V2.0.6
- Bio-Rad CFX96™ Real-time PCR Detection System with software V3.1

Note:

1. **Use film for the plate and cap for strip.**
2. **Use the dedicated PCR tube for the PCR machine.**

Process

This test process is optimized for DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit. Use of this kit is limited to qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

Overview

1. Sample collection

The kit can be used for nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum. Specimens should be collected, transported and stored according to standard procedures. Please refer to the CDC website for additional information: https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

***Reference. Sample Collection and Preservation (Source: Centers for Disease Control and Prevention)**

2. RNA Isolation

RNA should be extracted from nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes, bronchoalveolar lavage (BAL) fluid and sputum using the QIAamp Viral RNA Mini Kit (QIAGEN, catalog # 52904 or 52906) or MAgNa Pure 96 (Roche, 576 Extraction (06 543 588 001), External Lysis Buffer (06 374 913 001)). Other RNA extraction kits have not been qualified or validated.

- Perform the RNA extraction on the samples following the manufacturer's instructions for use (Qiagen). Recommended starting volume of samples is 140 µL. Extracted RNA should be eluted in a final volume of 60 µL.

- Perform the RNA extraction on the samples following the manufacturer's instructions for use (Roche). In the Roche kit, 310 µL of pre-aliquoted External Lysis Buffer is added to a 140 µL of sample (total input sample volume is 450 µL). Extracted RNA should be eluted in a final volume of 60 µL.

An External Positive Control must be processed in parallel with each batch of patient samples to monitor for RNA recovery and as a control for reverse transcription. Refer to the Quality Control Section below for information on how to prepare an appropriate External Positive Control.

3. Multiplex OneStep qRT-PCR

1) Please thaw all reagents on the ice. After vortex, spin down.
2) Prepare PCR Master Mix by adding the following reagents.
3) The amount of Master mix should be prepared by calculating overage corresponding to at least 1~2 reactions more than the number of samples and controls (including the PCR Positive Control (control template (2019-nCoV), NTC (Non-Template Control) and External Positive Control).
4) Mix master mix using vortex and spin down.

Component	1 rxn	4 rxns	5 rxns	6 rxns	10 rxns
OneStep qRT-PCR Buffer (2019-nCoV)	10 μℓ	40 μℓ	50 μℓ	60 μℓ	100 μℓ
OneStep qRT-PCR Enzyme mix (2019-nCoV)	2 μℓ	8 μℓ	10 μℓ	12 μℓ	20 μℓ
Primer & Probe Mixture (2019-nCoV)	3 μℓ	12 μℓ	15 μℓ	18 μℓ	30 μℓ
Total master mix volume	15 μℓ	60 μℓ	75 μℓ	90 μℓ	150 μℓ

Note:
Protect the Probe from the light. When the Probe is exposed to the light for a long time, fluorescence may be reduced and may affect the result.

5) Dispense 15 μℓ into a plate or strip tube suitable for the equipment using the manufactured master mix.
6) Add Template 5 μℓ .

Component	Volume
PCR master mix	15 μℓ
Template	5 μℓ
Total volume	20 μℓ

Note:
A PCR Positive Control (Control Template (2019-nCoV)) and NTC (Non-Template Control) should be included in each PCR run to check the normal function of the product and contamination of the laboratory environment. The PCR Positive Control uses Control Template (2019-nCoV) as template; NTC (Non-Template Control) uses RNase free water as template. An External Positive Control comprised of package viral RNA must also be tested in parallel with each batch of samples to monitor for RNA recovery and reverse transcription (refer to Quality Control section).

7) After sealing with cap or film, spin down.
8) Place the prepared PCR mixture on the instrument and proceed with PCR under the following conditions.

   * Refer to Appendix for device setup and Run

No.	Step	Temperature	Acquisition	Time	Cycles
1	Reverse transcription	50°C	-	15 min	1
2	Initial PCR activation	95°C	-	15 min	1
3	Denaturation	95°C	-	20 sec	45
4	Annealing/Extension	60°C	√	40 sec	

Analysis and results

1. Amplicon information

As shown in the following figure, you can check the detection of SARS-CoV-2 by comparing with the result of amplification of Control Template (2019-nCoV).

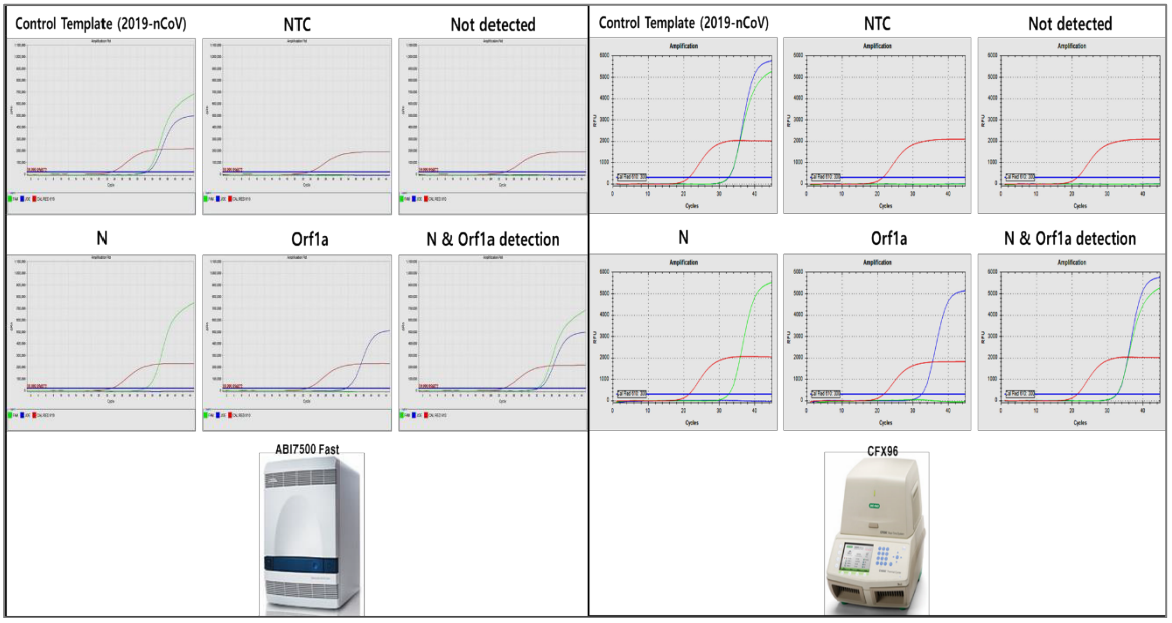

Figure 1. DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit Diagram.

Green is *N* gene (FAM), Blue is *Orf1a* gene (JOE/VIC), Red is PCRC (Texas Red/Cal Fluor Red 610)

2. Cut-off value

① If you are using ABI 7500 or ABI7500 FAST, you can check result of Ct value as follows:
   A. Plate and Film: Set the Threshold 20,000
   B. Tube and Cap: Set the Threshold 20,000

② If you are using CFX96™, you can check result of Ct value as follows:
   A. Plate and Film: Set the Threshold 300
   B. Tube and Cap: Set the Threshold 300

③ The amplification plots for the assay controls must satisfy the following conditions.

Control	N	Orf1a	PCRC	Expected Ct values of target
Non-Template Control	-	-	+	PCRC ≤26
PCR Positive Control or External Positive Control	+	+	+	N, Orf1a ≤40 PCRC ≤26

(*ABI 7500 / 7500 Fast set in "JOE" and "Texas Red", Bio Rad CFX96™ set in "VIC" and "Cal Fluor Red 610")
(*If the results show 40 < Ct ≤ 45, perform the experiment again.)

3. Result Interpretation for Patient Samples

Ct Value			Interpretation
N Gene	ORF1a	PCRC	
≤ 40	Any	Any	Positive
Any	≤ 40	Any	Positive
≤ 40	≤ 40	Any	Positive
> 40	None	Any	Inconclusive[1]
None	> 40	Any	Inconclusive[1]
> 40	> 40	Any	Inconclusive[1]
None	None	≤ 26	Negative
None	None	> 26 or None	Invalid[2]

[1] Repeat RT-PCR

[2] Repeat extraction and RT-PCR

Note:

1. **Even if the target is detected (Ct ≤40) and the PCRC is not detected, the result is still valid because:**
   - **If the sample is high concentration, PCRC may not amplify.**
   - **If PCR inhibitors are present, the PCRC may not amplify.**
2. **When the Non-Template Control test result is positive, all samples must be retested.**

※ PCRC (PCR Control)

Erroneous results may occur due to a variety of factors - for example, PCR mixture mix error, PCR condition error, PCR equipment use error etc. The PCR control is intended to monitor for the success of the PCR process. If the PCRC fails unexpectedly all experimental procedures and steps should be checked.

4. Required re-experiment

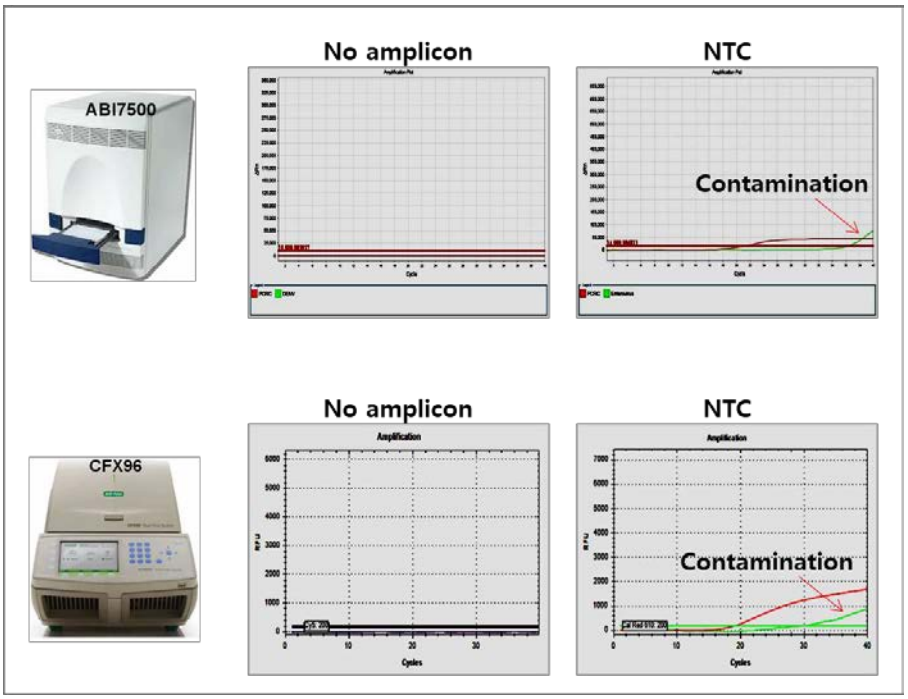

Figure 2. The failed result of curve pattern

Note:

If PCRC is not amplified, the purity of the RNA sample is not good. Thus, check it by dilute the sample (10 ~ 100 times) or extract RNA again.

This kit is for the detection of SARS-CoV-2 RNA. Clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status.

Quality Control

In accordance with ISO-certified Quality Management System of SolGent, each lot of DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit is tested against predetermined specifications to ensure consistent product quality. External controls are not provided with the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit. Quality control should be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

The following external control materials are available:

– AccuPlex™ SARS-CoV-2 Reference Material Kit (Cat. No. 0505-0126)

Positive External Controls should be prepared by diluting the stock of virus particles in PBS to a final concentration of 1,000 copies/200 µL. External Positive Controls must be processed like patient samples to monitor RNA extraction, reverse transcription, PCR amplification and detection.

At least one External Positive Control must be processed with every batch of patient samples. The expected result must be obtained with the External Positive Control, as well as the Positive (Template) and Negative (Non-Template) PCR Controls in order to interpret the results obtained with patient samples.

Limitations

- The use of this assay as an in vitro diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may lead to erroneous results.

- Patient results should only be interpreted if the results from the assay controls are valid.

- The performance of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit assay was established using sputum and contrived nasopharyngeal swab samples. Oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid are also considered acceptable specimen types for use with DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit. Please refer to the FDA FAQs on Diagnostic Testing for SARS-CoV-2 for additional information regarding acceptable specimen types for detection of SARS-CoV-2.

- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 target sequences
  - Failure to follow the instructions for use

- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Specimen mix-up
  - RNA contamination during RT-PCR set-up

- The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.

- A positive result for either the N or ORF1a targets indicates the detection of nucleic acid from SARS-CoV-2.

- Nucleic acid may persist even after the virus is no longer viable.

- Laboratories are required to report all positive results to the appropriate public health authorities.

Conditions of Authorization for the Laboratory

The DiaPlexQ™ Novel Coronavirus (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/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#2019-ncov.
However, to assist clinical laboratories running the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit will include with result reports of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit, 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 DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit will use the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit as outlined in the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit 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 perform the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit are not permitted.

C. Authorized laboratories that receive the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the DiaPlexQ™ Novel Coronavirus (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 test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and SolGent Co., Ltd. local technical support center (via email: global@solgent.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 test must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

G. SolGent Co., Ltd., authorized distributors, and authorized laboratories using the DiaPlexQ™ Novel Coronavirus (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] The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

Trouble Shooting

Problem	Possible Causes	Solution
No or weak PCR product fluorescent	Wrong Storage condition	Check storage temperature for the reagents and obtain new reagents if needed
	Too short time for Enzyme activation in PCR reaction	Check that the "initial PCR activation" is set for 15min at 95℃
	Expired shelf life	Check the expiration date and obtain new reagents if needed
	Primer and probe degraded	Check primers & probes by swapping with a new primer and probe mixture from the same kit lot.
	Low template quality	Check template quality using spectrophotometer
	Inhibitors in Template	Check the processing conditions for the sample. Repeat extraction as appropriate.
	Inappropriate Nucleic acid preparation	Check the concentration of nucleic acid. Re-extract the nucleic acid if needed.
	Template degradation	Re-extract template
	Reagents stored at room temperature	Do not leave reagents at room temperature for extended periods of time. Obtain new reagents as needed.
	Deactivation of Plate read	Re-test after activating plate read in the steps provided in the IFU when setting PCR conditions in the PCR machine
	Unassigned Fluorophore in sample well	Assign the correct fluorophore following the IFU and reanalyze the data.
Non-specific PCR Amplification	Contamination of PCR mixture	Check to confirm that the laboratory environment and equipment have not been contaminated. Clean as needed.
	Contamination from the extraction process	If environment and equipment have not been contaminated, replace RNA extraction PCR reagents
	Contamination of Water	Obtain new nuclease free water
False positive / PCR product with non-template control(NTC)	Cross-contamination	Use filter tips, screw-cap tubes and latex gloves. Perform assay set-up in a hood in a clean environment.
Conflicting or unexpected results for different optical channels	Pipette volume error	Check the Pipettes and calibrate as needed
	Cross contamination	Be careful when you add samples to the PCR tubes
	If there are foreign objects on the PCR tubes or caps	Remove any debris with a soft cloth before performing the PCR.
No PCR product with positive control or false negative	Template degradation	Do no repeatedly freeze-thaw the positive control (plasmid DNA)
	Incorrect storage	Check storage condition for kit and use a new kit if needed
	Inappropriate Nucleic acid preparation	Check the concentration of nucleic acid. Re-extract a fresh aliquot of the sample if needed
	Incorrect PCR mixture (primer & premix) volume	Check the volumes used for the mixture in case of pipetting error
	Storage of the reagents at room temperature	Do not store the reagents at room temperature and obtain new reagents if needed

Performance Characteristics

1. Limit of Detection (LoD) – Analytical Sensitivity

The preliminary LoD was established by testing serial dilutions of SARS-CoV-2 packaged viral RNA using the ABI 7500 Fast system. The samples of serial dilutions (4,000 copies/mL, 400 copies/mL, 200 copies/mL, 40 copies/mL) were prepared by spiking the quantified SARS-CoV-2 packaged viral RNA into negative respiratory clinical matrices (nasopharyngeal swab and sputum). Each replicate was extracted using the Qiagen QIAamp Viral RNA Mini Kit. For both matrices, the lowest target level at which all five replicates produced positive results was 200 copies/mL. The LoD was confirmed by testing 20 replicates at the estimated LoD concentration. All 20/20 test results with both nasopharyngeal swabs and sputum were positive. The LoD of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit with nasopharyngeal swabs and sputum was confirmed to be 200 copies/mL.

N gene: Preliminary LoD determination with SARS-CoV-2 packaged viral RNA

Specimen	Copies/mL	Results	Ct					Mean Ct (n=5)	SD
Nasopharyngeal swab	4,000	5/5	29.2	29.0	29.4	29.3	29.5	29.3	0.192
	400	5/5	32.8	32.5	32.4	32.3	32.5	32.5	0.187
	200	5/5	34.0	34.6	35.2	35.9	34.9	34.9	0.705
	40	2/5	N/D	36.7	N/D	N/D	36.4	36.6	0.212
Sputum	4,000	5/5	30.1	30.2	29.6	28.9	29.9	29.7	0.522
	400	5/5	32.4	33.4	32.8	32.6	33.2	32.9	0.415
	200	5/5	33.7	34.0	34.5	34.0	35.8	34.4	0.834
	40	4/5	34.9	34.0	N/D	34.8	37.0	35.2	1.282

Orf1a: Preliminary LoD determination with SARS-CoV-2 packaged viral RNA

Specimen	Copies/mL	Results	Ct					Mean Ct (n=5)	SD
Nasopharyngeal swab	4,000	5/5	31.9	31.5	31.5	31.8	31.8	31.7	0.187
	400	5/5	35.6	35.7	35.7	35.3	34.8	35.4	0.383
	200	5/5	36.8	37.9	37.2	37.0	38.0	37.4	0.540
	40	5/5	39.4	39.2	36.2	37.6	38.0	38.1	1.301
Sputum	4,000	5/5	31.7	31.8	32.1	31.9	32.0	31.9	0.158
	400	5/5	34.5	35.0	34.9	35.5	34.7	34.9	0.377
	200	5/5	37.7	38.0	38.1	37.7	38.3	38.0	0.261
	40	4/5	36.6	37.4	35.5	35.3	N/D	36.2	0.983

N gene: Final LoD confirmation with SARS-CoV-2 packaged viral RNA

Specimen	Copies/mL	Results	Ct					Mean Ct (n=20)	SD
Nasopharyngeal swab	200	20/20	33.5	33.5	34.6	33.3	33.5	33.3	0.440
			33.0	33.3	33.5	33.0	33.3		
			33.5	32.7	32.5	33.4	33.2		
			33.9	33.5	33.3	33.2	33.1		
Sputum	200	20/20	32.5	34.1	33.3	32.5	33.2	33.2	0.469
			33.1	33.5	33.5	33.4	33.6		
			32.7	33.1	32.7	32.5	33.4		
			33.5	33.4	32.9	34.0	32.9		

Orf1a: Final LoD confirmation with SARS-CoV-2 packaged viral RNA

Specimen	Copies/mL	Results	Ct					Mean Ct (n=20)	SD
Nasopharyngeal swab	200	20/20	34.7	34.0	34.9	36.0	34.4	34.5	0.588
			34.1	34.7	35.2	34.8	34.0		
			34.6	35.4	34.0	34.2	34.4		
			33.8	34.3	35.3	33.8	34.3		
Sputum	200	20/20	33.5	34.4	34.4	33.6	33.8	34.2	0.537
			34.7	34.5	34.5	34.2	35.3		
			33.9	33.9	33.7	33.9	33.8		
			34.8	33.7	33.7	34.2	35.3		

Additional testing was performed with both nasopharyngeal swab matrix and sputum which showed that the LoD of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit using the ABI 7500 system or Bio-Rad CFX96 system was similar to that obtained with the ABI 7500 Fast system. Furthermore, the LoD of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit using the MagNa Pure 96 was found to be similar to that when using the Qiagen QIAamp Viral RNA Mini Kit. The results from these studies demonstrated that these alternative PCR instrument systems and extraction technologies may be used interchangeably.

2. Inclusivity – In silico Analysis

The assays were mapped to 17,228 SARS-CoV-2 genomes in GISAID databases as of May 7, 2020. 91 sequences (0.53%) exhibited a single mismatch with one of the primers or the probe for the N gene and that there were two additional sequences that had single base mismatches with both the N gene reverse primer and the probe. None of the sequences with mismatches to the N gene primers/probe had any mismatches with the primers and probe for the ORF1a target region. For ORF1a, there were 138/17228 (0.80%) that exhibited a single base mismatch with one of the primers or the probe used in the kit. Of these, 126 had no mismatches with the N gene primers and probe and 12 had low quality sequence information in the N gene region.

3. Cross-reactivity

To demonstrate the analytical specificity of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit testing was performed using high concentrations (>10^5 genomic equivalents/mL) of purified RNA or DNA from organisms and viruses that may be found in the respiratory tract. (Table). No cross reaction was observed.

No.	Viruses / Bacteria	Ct Value
1	Parainfluenza I	N/A
2	Parainfluenza II	N/A
3	Parainfluenza III	N/A
4	Parainfluenza IV	N/A
5	Influenza A	N/A
6	Influenza B	N/A
7	Adenovirus	N/A
8	Respiratory syncytial virus A	N/A
9	Respiratory syncytial virus B	N/A
10	Rhino 8, A	N/A
11	Bocavirus	N/A
12	Metapneumovirus	N/A
13	Beta Coronavirus OC43	N/A
14	Alpha Coronavirus 229E	N/A
15	Enterovirus	N/A
16	*Acinetobacter baumannii*	N/A
17	*Bordetella parapertussis*	N/A
18	*Bordetella pertussis*	N/A
19	*Chlamydophila pneumoniae*	N/A
20	*Haemophilus influenza*	N/A

No.	Viruses / Bacteria	Ct Value
21	*Klebsiella pneumoniae*	N/A
22	*Legionella pneumophila*	N/A
23	*Moraxella catarrhalis*	N/A
24	*Mycoplasma pnemoniae*	N/A
25	*Pseudomonas aeruginosa*	N/A
26	*Serratia marcescens*	N/A
27	*Staphylococcus aureus*	N/A
28	*Stenotrophomonas maltophilia*	N/A
29	*Streptococcus pneumoniae*	N/A
30	*Mycobacterium abscessus*	N/A
31	*Mycobacterium avium*	N/A
32	*Mycobacterium bovis*	N/A
33	*Mycobacterium chelonae*	N/A
34	*Mycobacterium intracellulare*	N/A
35	*Mycobacterium kansasii*	N/A
36	*Mycobacterium scrofulaceum*	N/A
37	*Mycobacterium tuberculosis*	N/A
38	Human total RNA (10ng/µl)	N/A
39	SARS CoV-2 N, Orf1a (Transcript RNA)	Detection

N/A: Not applicable

4. Precision Test

In Vitro transcript RNA containing the target genes was used to evaluate the precision of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit by testing at concentrations of 1 X 10^5, 1 X 10^3, 1 X 10^1 copies per reaction by addition directly to the PCR mixture without nucleic acid extraction. The study was performed over 5 days by testing 4 replicates with each of 2 reagent lots once a day (5 days x 4 replicates x 2 reagent lots = 40 replicates per target level). As a result, the standard deviation of for the Ct values for the N and Orf1a targets between lots & days was less than 1 and the coefficient of variation was less than 5%.

Precision test result between Lot					
Target gene	Copies No.	Lot 1 Average Ct value	Lot 2 Average Ct value	Standard Deviation between Lot	%CV between Lot
N	1 X 10^5	19.9	20.1	0.143	0.718
	1 X 10^3	27.9	27.9	0.019	0.068
	1 X 10^1	35.1	35.7	0.412	1.164
Orf1a	1 X 10^5	20.9	20.9	0.012	0.060
	1 X 10^3	28.5	28.6	0.096	0.335
	1 X 10^1	35.5	35.8	0.193	0.543

Repeatability test results between test days				
Target gene	Copies No.	Average of Ct value	Standard Deviation	%CV
N	1 X 10^5	20.0	0.382	1.914
	1 X 10^3	27.9	0.429	1.537
	1 X 10^1	35.4	1.487	4.205
Orf1a	1 X 10^5	20.9	0.252	1.206
	1 X 10^3	28.6	0.520	1.821
	1 X 10^1	35.6	1.057	2.967

5. Clinical Evaluation

A clinical evaluation study was performed to evaluate the performance of the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit using contrived nasopharyngeal swab specimens and sputum.

5-1) Nasopharyngeal swab Clinical Evaluation Study

Thirty (30) contrived positive specimens and a thirty (30) negative specimens were tested. Positive samples were contrived by spiking known concentrations of SARS-CoV-2 packaged viral RNA, into SARS-CoV-2 negative matrices.

The positive and negative percent agreements between the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit and the expected results with nasopharyngeal swabs are shown below.

Clinical Evaluation Study of Contrived Positive Nasopharyngeal swab

Final RNA Concentration in Sample	Number of Samples Tested	SARS-CoV-2-N		SARS-CoV-2- Orf1a		Overall SARS-CoV-2 Result	% Positivity
		Mean Ct Value	% Agreement (#Pos or Neg) /Total	Mean Ct Value	% Agreement (#Pos or Neg) / Total		
2X LoD	20	33.1	100% 20/20	34.1	100% 20/20	Positive	100%
3X LoD	5	32.7	100% 5/5	33.5	100% 5/5	Positive	100%
4X LoD	5	32.1	100% 5/5	34.1	100% 5/5	Positive	100%
Negative	30	N/D	100% 30/30	N/D	100% 30/30	Negative	0%

5-2) Sputum Clinical Evaluation Study

Thirty (30) SARS-CoV-2 positive and a thirty (30) SARS-CoV-2 negative clinical sputum specimens were tested. The SARS-CoV-2 status of the specimens was determined using an alternative real-time PCR method, that is FDA-authorized for emergency use.

- 30 positive sputum specimens
- 30 negative sputum specimens

Sputum Clinical Evaluation Study

Patient sample	Diagnosis positive	Diagnosis negative	Total
Test positive	30	0	30
Test negative	0	30	30
Total	30	30	60
		%	% Agreement [95% CI]
PPV		100	88.65%-100%
NPV		100	88.65%-100%

Compared to another molecular method, with sputum specimens, the DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit showed positive and negative percent agreement of 100% (95% CI: 88.65% to 100%). See Table above for summary of clinical results.

6. 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 extraction method and instrument used were Qiagen QIAamp Viral RNA Mini Kit and ABI 7500 Fast system. The results are summarized in Table below.

Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal swab	1.8×10^4 NDU/mL	N/A
MERS-CoV		N/A	ND

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

Appendix

■ Applied Biosystems™ 7500 / 7500Fast Real-Time PCR Instrument System Set up and Run

1. Click 'Advanced Setup' on the main screen.

Figure 3. Main

2. Enter the file name (or Experiment Properties screen).

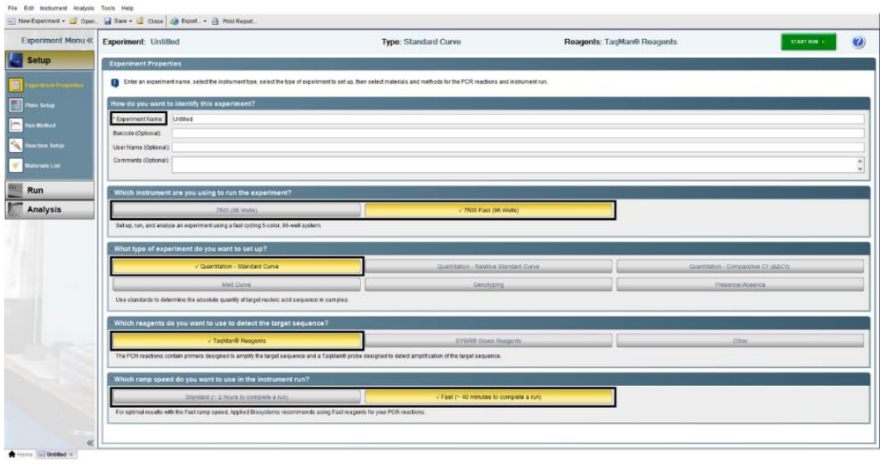

Figure 4. Experiment Properties

2-1. Fill in "Experiment Name"

2-2. "Which instrument are you using to run the experiment?"

→ Check 7500 (96 Wells) or 7500 Fast (96 Wells)

2-3. "What type of experiment do you want to set up?"

→ Check Quantitation – Standard Curve

2-4. "Which reagents do you want to use to detect the target sequence?"

→ Check TaqMan® Reagents

2-5. "Which ramp speed do you want to use in the instrument run?"

→ Check Standard (~ 2 hours to complete a run) or Fast (~40 minutes to complete a run)

3. At the 'Define Targets and Samples' Tap in Plate Setup screen, please set up as follows.

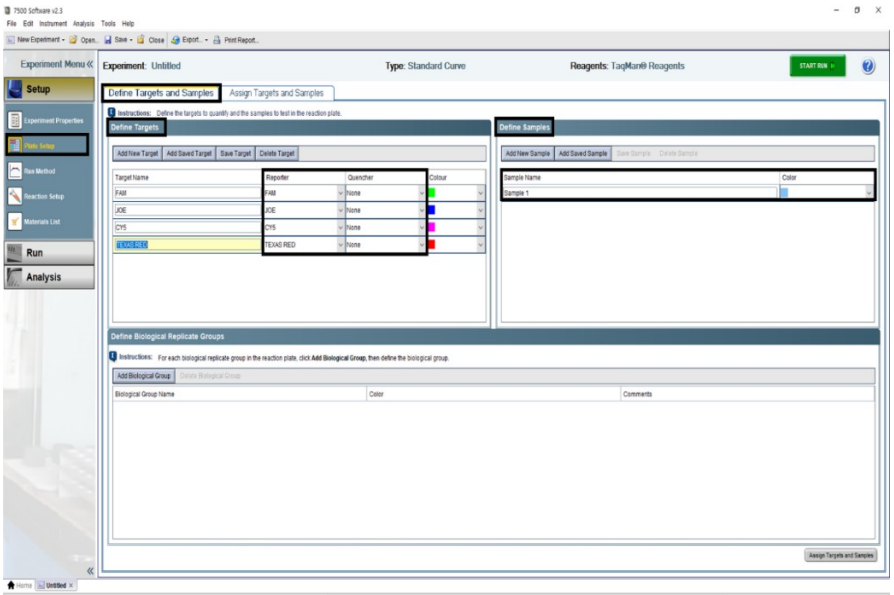

Figure 5. Plate Setup - Define Targets and Samples

3-1. Click 'Add New Target' at Define Targets. Setup 'Reporter' and 'Quencher' as follows:
(Target Name and Color can be setup randomly.)

Reporter	Quencher
FAM	none
JOE	none
Texas Red	none

The Kit does not include a "Reference dye"

(E.g. Set up the reference dye to "None" in the ABI 7500 / 7500 Fast program)

3-2. If you want to fill out sample name, you can assign randomly at 'Define Samples'.

4. At 'Assign Targets and Samples' Tap in 'Plate Setup' screen, please set up as follows.

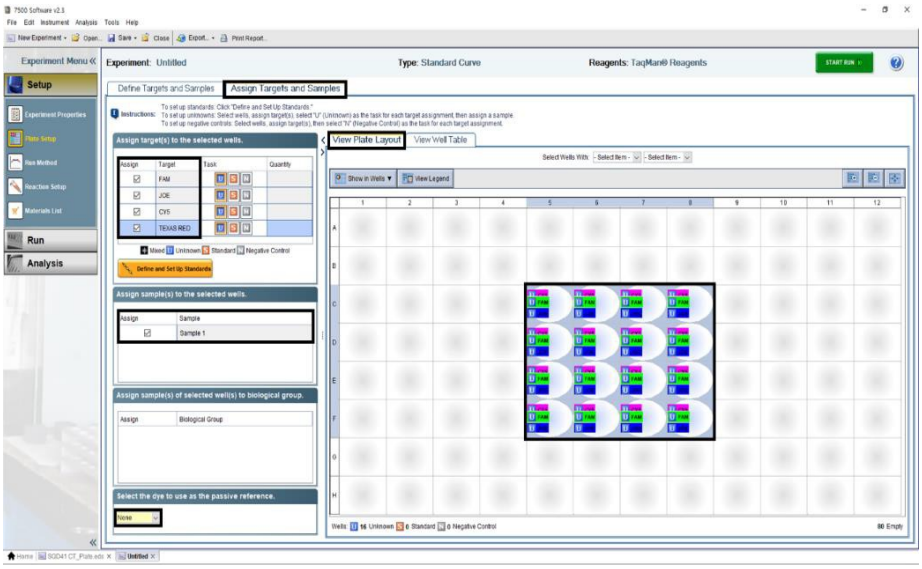

Figure 6. Plate Setup - Assign Targets and Samples

4-1. "View Plate Layout" Select well according to the position of the PCR mixture reaction solution.

4-2. "Assign target(s) to the selected wells" Select Target (3-1).

4-3. "Assign samples(s) to the selected wells" Select Sample (3-2).

4-4. "Select the dye to use as the passive reference" Select None.

5. Set the PCR temperature condition as follows, enter the reaction volume as 20 ㎕ and click 'Start Run'.

No.	Step	Temperature	Acquisition	Time	Cycles
1	Reverse transcription	50°C	-	15 min	1
2	Initial PCR activation	95°C	-	15 min	1
3	Denaturation	95°C	-	20 sec	45
4	Annealing/Extension	60°C	√	40 sec	

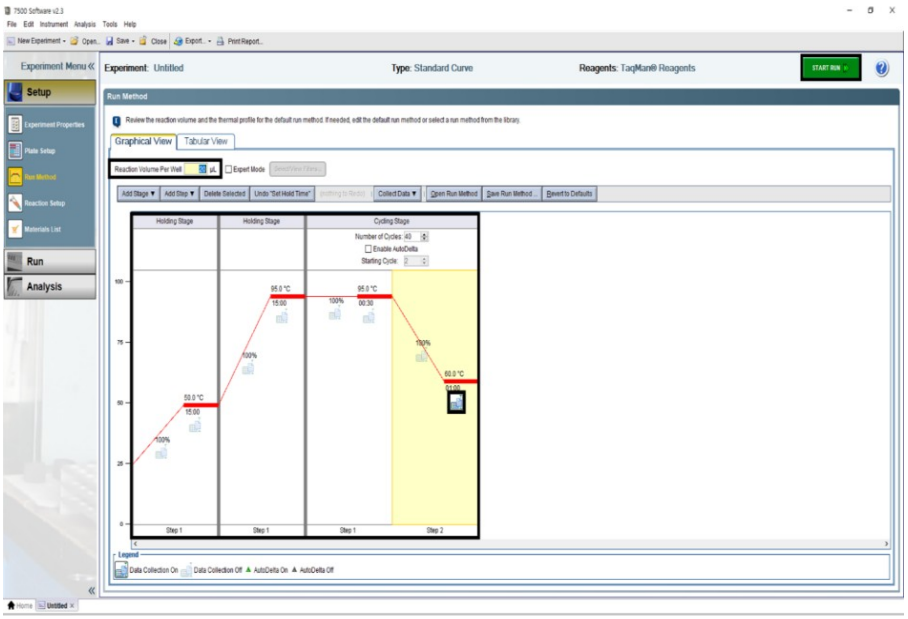

Figure 7. Run Method

Note: In Step 6, check Collect Data on Hold to collect data.

6. Select 'START RUN' and set the location where the data will be saved.

■ Bio-Rad CFX96™ System Setup and Run

1. Turn on the instrument.
2. Run Bio-Rad CFX Manager.
3. Click 'File' → 'New' → 'Protocol'.

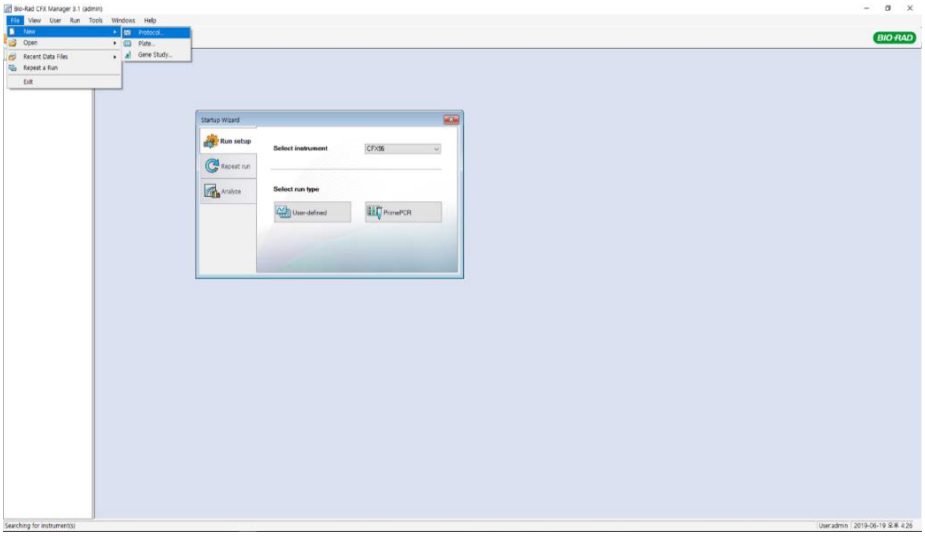

Figure 8. Main

4. In the Protocol Editor screen, enter Sample Volume 20 ㎕, set the PCR Condition, and click 'OK'

No.	Step	Temperature	Acquisition	Time	Cycles
1	Reverse transcription	50°C	-	15 min	1
2	Initial PCR activation	95°C	-	15 min	1
3	Denaturation	95°C	-	20 sec	45
4	Annealing/Extension	60°C	√	40 sec	

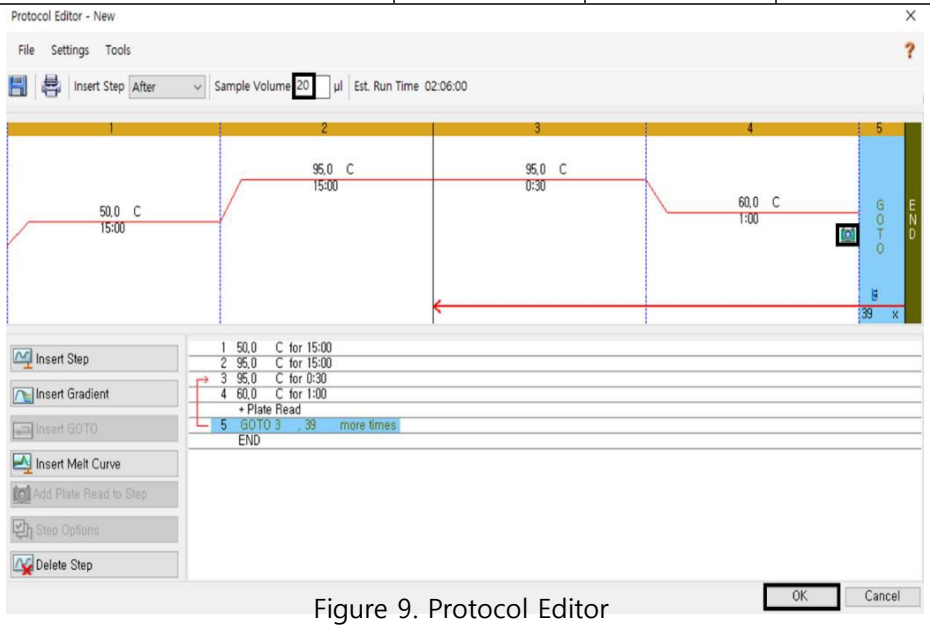

Figure 9. Protocol Editor

Note: In Step 4, check 'Collect Data on Hold' to collect data.

5. Click 'Create New' in the plate tap. In 'Plate Editor' screen, click 'Select Fluorophores' and setup fluorophore.

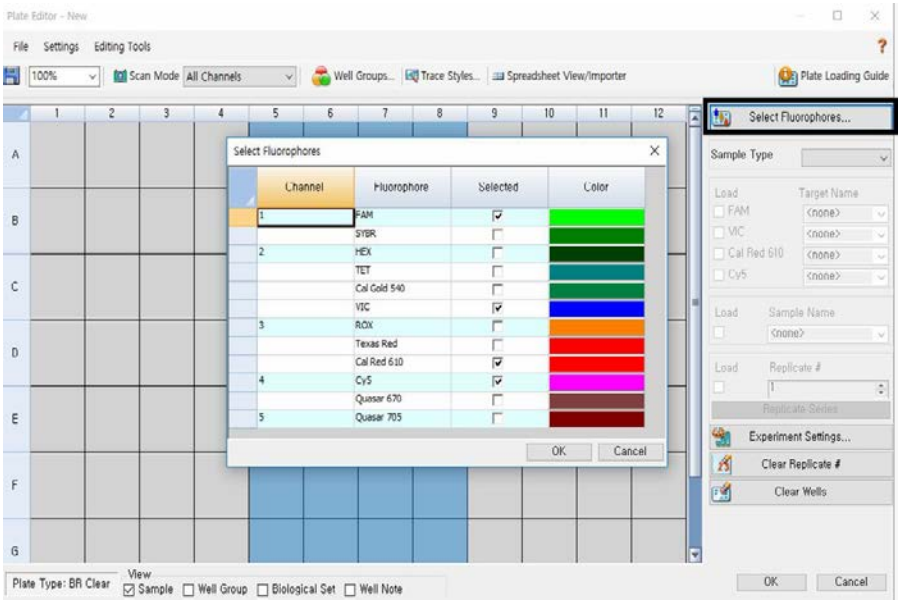

Figure 10. Plate Editor - 1

Fluorophore
FAM
VIC
Cal Fluor Red 610

6. After selecting well according to the position of PCR mixture reaction solution, designate 'Sample Type' and 'Fluorophore'.

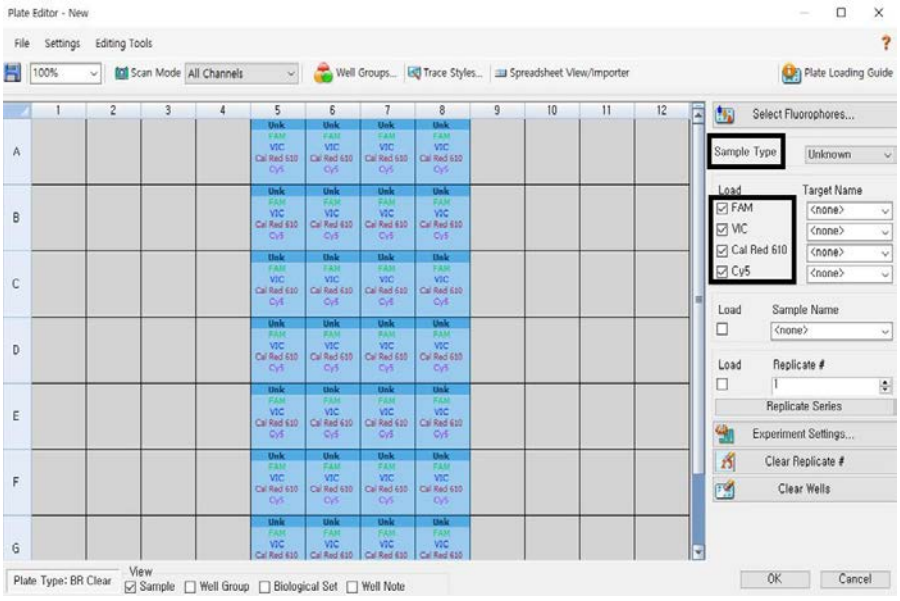

Figure 11. Plate Editor - 2

7. Settings → Plate Type → click 'BR White' or 'BR Clear' according to the type you are using.

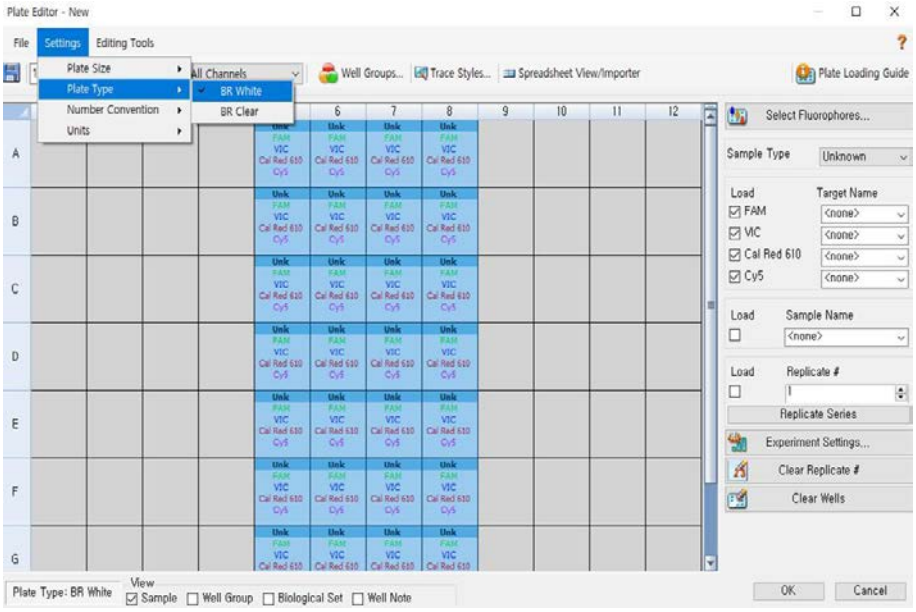

Figure 12. Plate Editor - 3

8. On the 'Start Run' tab in 'Run Setup', click 'Close Lid' to close the lid of the instrument, select the active 'Start Run' and set the location where the data will be saved.

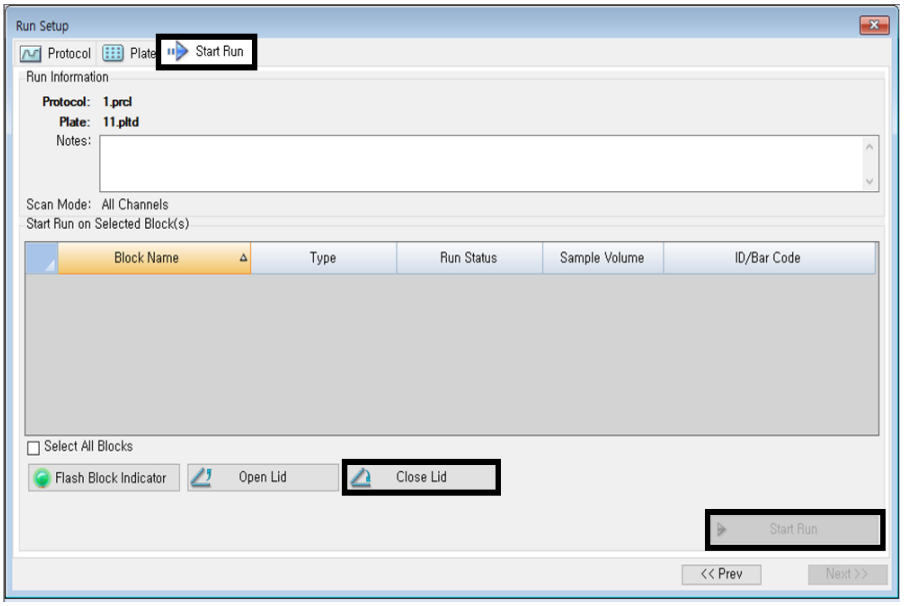

Figure 13. Run Setup

■ Applied Biosystems™ 7500 / 7500Fast Real-Time PCR Instrument System setup for result analysis

1. After Real-Time PCR is finished, set 'Plot Settings' on the 'Amplification Plot screen' as below and select 'Analysis Settings'.

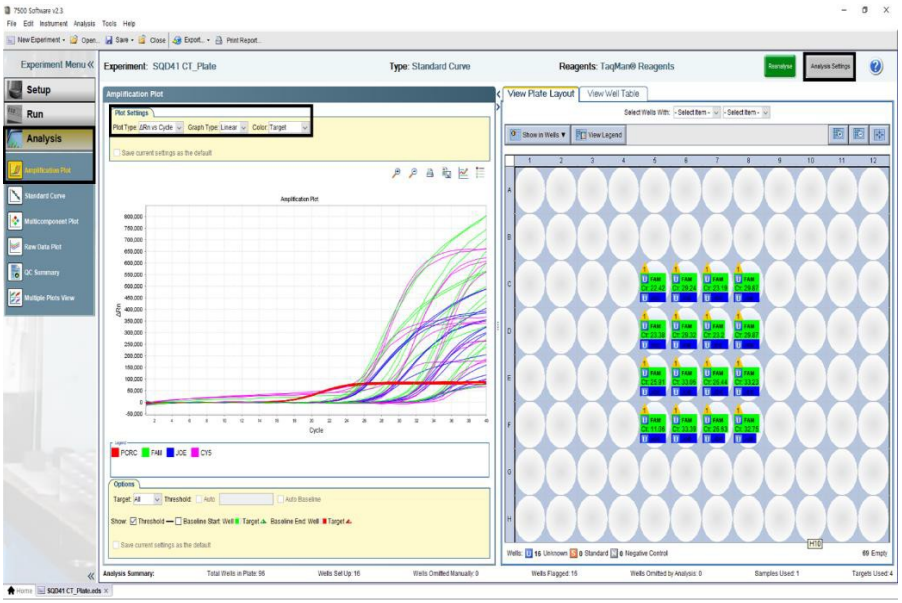

Figure 14. Amplification Plot

1-1. "Plot Type" ΔRn vs Cycle / "Graph Type" Linear / "Color" Target

2. In 'Analysis Settings', specify the Threshold value for each Fluorophore, and then click 'Apply Analysis Settings'. * Threshold: 20,000 (Plate / Strip tube)

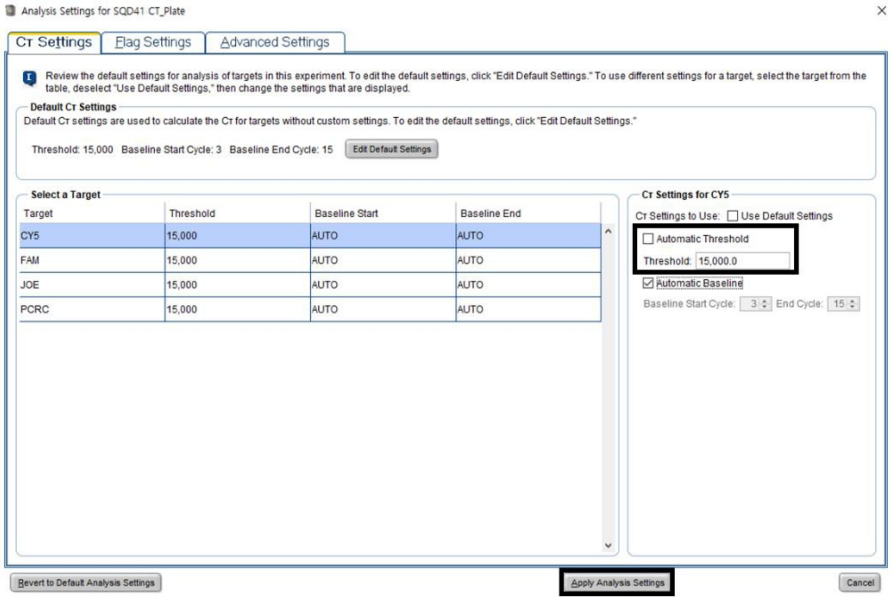

Figure 15. Analysis Settings

3. Interpret the results by referring to the result analysis.

■ Bio-Rad CFX96™ System Setup for result analysis

1. After Real-Time PCR is finished, check 'Fluorophore' in Data Analysis screen and click Settings → Baseline Threshold.

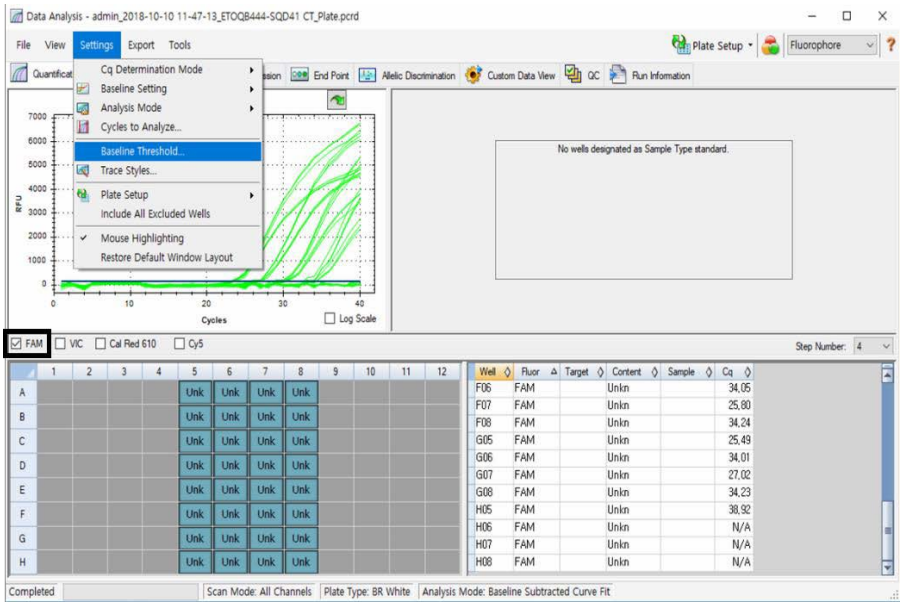

Figure 16. Data Analysis

2. In the Baseline Threshold Screen, specify the Threshold value for each fluorophore and click OK.
   * Threshold: 300 (Plate / Strip tube)

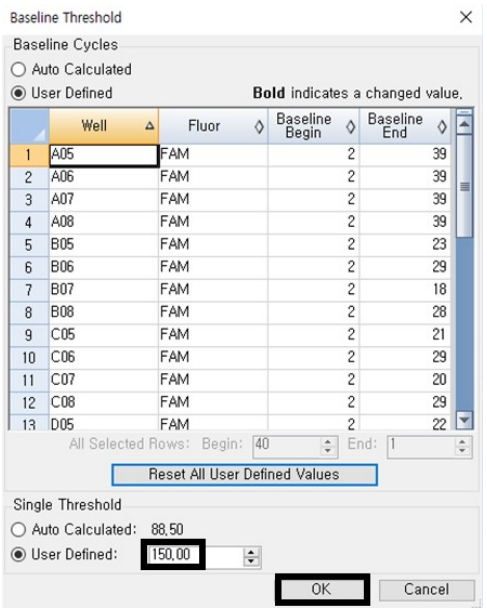

Figure 17. Baseline Threshold

3. Interpret the results by referring to the result analysis.

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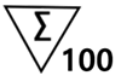
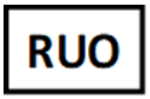
Symbols

Symbol	Used for	Example of Usage
	Temperature limit	
	Use-by date	
	Batch code	
	Catalog number	
	Manufacturer	
	Date of Manufacture	
	Serial number	
	Contains sufficient for <*n*> tests	

Symbol	Used for
	Caution
	Consult instructions for use
	Research use only
	CE mark
	*In vitro* diagnostic medical device
	Authorized representative in the European Community
	Positive control
	Keep away from sunlight
	Prescription Use Only

Ordering Information

Cat. No.	Name	Size
SQD52-K100	DiaPlexQ™ Novel Coronavirus (2019-CoV) Detection Kit	100 reactions/Kit

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