REF M22MD100M

1copy™ COVID-19 qPCR Multi Kit

Σ 100

IVD CE

For *in vitro* diagnostic use
Prescription Use Only

Instrutions for Use

Rev. Date : May 25, 2020
Doc.no. DR-M22-6007-E-07

1. Description

1copy™ COVID-19 qPCR Multi Kit provides reagents for real-time RT-PCR that specifically targets the E (Envelope) gene and the RdRp (RNA dependent RNA polymerase) gene for detection of SARS-CoV-2, in specimens obtained from nasopharyngeal swab, anterior nasal swab, mid-turbinate nasal swab and oropharyngeal swab as well as nasopharyngeal wash/aspirate and nasal aspirate.

2. Intended Use

1copy™ COVID-19 qPCR Multi Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, oropharyngeal, anterior nasal, mid-turbinate nasal swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests or by similarly qualified non-U.S. laboratories.

Results are used to identify the presence of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during an acute phase of infection. Positive results indicate presence of SARS-CoV-2 RNA, but clinical correlation with patient history and other diagnostic information are necessary to rule out a patient's 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.

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 1copy™ COVID-19 qPCR Multi 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.

3. Principle of the Assay

The 1copy™ COVID-19 qPCR Multi Kit is a real-time reverse transcriptase polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) are designed according to the "WHO interim guidance for laboratory testing for 2019 novel coronavirus (2019-nCoV) in humans". This kit is based on TaqMan probe real-time fluorescent PCR technology. Upper respiratory specimens (nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate swabs, nasopharyngeal wash/aspirates and nasal aspirate specimens) are extracted using QIAamp Viral RNA Mini Kit (QIAgen). After extraction, the purified nucleic acid is first generated into cDNA by reverse transcriptase, then amplified by Taq DNA polymerase in the rRT-PCR instrument.
During the PCR amplification, the 5' nuclease activity of Taq DNA polymerase causes the degradation of the TaqMan probe, allowing the reporter dye to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the rRT-PCR instrument: FAM channel qualitative detection of SARS-CoV-2 E gene in E gene assay mixture (first well) and SARS-CoV-2 RdRp gene in RdRp gene assay mixture (second well), and Texas Red channel detection of internal positive control in E gene assay mixture (first well). The kit uses dUTP and UNG enzymes to prevent contamination of amplified products.

E gene assay mixture (1st well)		RdRp gene assay mixture (2nd well)	
Target	Channel	Target	Channel
E gene	FAM	RdRp gene	FAM
IPC	Texas Red		

4. Kit Contents (Materials Provided)

Kit contents	Cap color	Volume (100 Test)
Master mix	Red	2 x 1000 µℓ
Primer/Probe mix 1 (E gene, IPC)	Brown (Amber tube)	100 µℓ
Primer/Probe mix 2(RdRp gene)	Brown (Amber tube)	100 µℓ
Control 1 (E gene)	Yellow	100 µℓ
Control 2 (RdRp gene)	Yellow	100 µℓ
DEPC DW	Clear	1000 µℓ

※ Control 1 for E gene and Control 2 for RdRp gene are positive controls.
※ DEPC DW (Diethylpyrocarbonate-treated water; nuclease-free water) is used as a negative control.

5. Materials Required but Not Provided

* Provided with the kit (please see kit contents, section 4)

- RNase/DNase free consumables (disposable latex or vinyl gloves)
- Filter tips
- 0.5㎖ or 0.2㎖ PCR tubes or 96-well PCR plates compatible with PCR instrument manufacturer's instructions
- 1.5㎖ micro tubes
- Sealing film
- Ice or cooling/cold block
- Microliter pipettes (1~10µℓ, 10~100µℓ, 100~1000µℓ)
- Mini centrifuge (0.2㎖/0.5㎖ tubes, 10,000 rpm) or Benchtop centrifuge (1.5 mL microcentrifuge and 96 well plate centrifuge) with rotor for 0.2㎖/0.5㎖ reaction tubes (capable of attaining 10,000 rpm), vortexer
- Sample collection and sample preservation buffer (Puritan UniTranz-RT 3㎖ Filled Vial w/ Elongated & Ultrafine Flock Swabs (Cat No. UT-367))
- Real-time PCR instrument (See Section 6 below)
- QIAamp Viral RNA Mini Kit (QIAGEN, Cat no.52904)
- Ethanol (96~100%)

6. Compatible Real-time PCR Instruments

- **Light Cycler 480**
  (Roche, Product No. 05015278001, Software version 1.5)
- **Rotor-Gene Q 5plex HRM**
  (Qiagen, Product No. 9001580, Software version 2.3.4)
- **Applied Biosystems Quantstudio5**
  (Thermo Fisher Scientific, Product No. A28134, Software version 1.4.3)
- **Applied Biosystems 7500 Real-Time PCR Instrument system**
  (Thermo Fisher Scientific, Product No. 4345241, Software version 2.0.6)
- **CFX96™ Real-Time PCR Detection system**
  (BIO-RAD, Product No. 1854095-IVD, Software Bio-Rad CFX Maestro version 1.1)

7. Warnings and Precautions

- 1copy™ COVID-19 qPCR Multi Kit is for *in vitro* diagnostic use only.
- Follow standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious, following safe laboratory procedures. Refer to the Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV 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.
- Please read the Instructions for Use carefully prior to testing. 1copy™ COVID-19 qPCR Multi Kit, which is only for use with a prescription, as an *in vitro* diagnostic test. Each step of the process, from specimen collection, storage, transportation, and to laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.
- False positive and false negative results can appear due to poor specimen quality, improper specimen collection, improper transportation, improper laboratory processing, and/or limitation of the testing technology. The operator should understand the principles of the procedures in advance, including its performance limitations, to avoid potential mistakes.
- Separate laboratory areas, dedicated to performing predefined procedures of the assay, are required. a) 1st Area: Preparation Area—Prepare testing reagent: b) 2nd Area: specimen processing—Process the specimen and controls: c) 3rd: Amplification Area—PCR conducted.
- All materials used in one area should remain in that area and should not be moved or used in other areas. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected immediately.
- All contents of this package are prepared and validated for the intended testing purpose. Replacement of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix components from different lots.
- This product is intended for professional use only and should be handled by clinical laboratory personnel specifically trained in the techniques of real-time PCR and *in vitro* diagnostic procedures for use in clinical specimens.
- Do not use expired components.
- Wear appropriate protective clothing, disposable gloves and protective gloves.
- Use filter pipette tips to avoid contamination.
- Do not mix reagents from different lots of 1copy™ COVID-19 qPCR Multi Kit.
- Minimize the temperature difference of the components.
- Thaw necessary components just before using and promptly place back in freezer after use.
- Use thawed contents after gently mixing and spinning down.
- Prepare mixtures of qPCR within a cooling/cold block or on ice.
- Discard unused reagents, waste and control according to laboratory safety rules and guidelines.
- In case of contact with eyes, rinse immediately with water.
- Be sure to deposit samples, using a pipette, directly into the reaction mix in PCR tubes. Do not deposit samples with the pipette to the inside plate well wall. The plates should be sealed immediately after the addition of sample. Following the amplification protocol, PCR plates should be placed into a sealable plastic bag for autoclaving and decontamination.
- Be sure not to introduce any foam or bubbles into the tubes when aliquoting Nucleic Acid reaction Mix. All PCR plates should be sealed prior to centrifugation and subsequent loading into the thermocycler to avoid any possible leakage and contamination.
- All lab workbench and supplies should be cleaned and disinfected regularly using 75% Ethanol or UV light.
- All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The used centrifuge tubes and pipette tips should be discarded in waste bin with bleach and after decontamination.
- Avoid exposure of the Primer/Probe Mixture to light.
- Even if the test results of this product are 'positive', it should be interpreted by an experienced specialist and various results such as the patient's symptoms should also be reviewed.
- Even if the test results of this product are 'negative', it should be interpreted by an experienced specialist and various results such as the patient's symptoms should also be reviewed, without excluding infection.

8. Reagent Storage and Handling

- Store the kit below -20°C.
- Expiration date for kit is indicated on the package.
- Freezing and thawing is limited to 5 times.
- Minimize the temperature difference of the components.
- Thaw necessary components just before using and promptly place back in freezer after use.

9. Procedure

9.1 Specimen collection, transport and storage

Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13 (Clinical and Laboratory Standards Institute) may be referenced as an appropriate resource.

Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.

Refer to the CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) https://www.cdc.gov/coronavirus/SARS-CoV-2/lab-biosafety-guidelines.html.

Follow specimen collection devices manufacturer instructions for proper methods.

Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media or universal transport media. The swab specimens for testing can be stored up to 72 hours at 2-8°C, with long-term storage at -70° C or below.

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens to the testing laboratory.

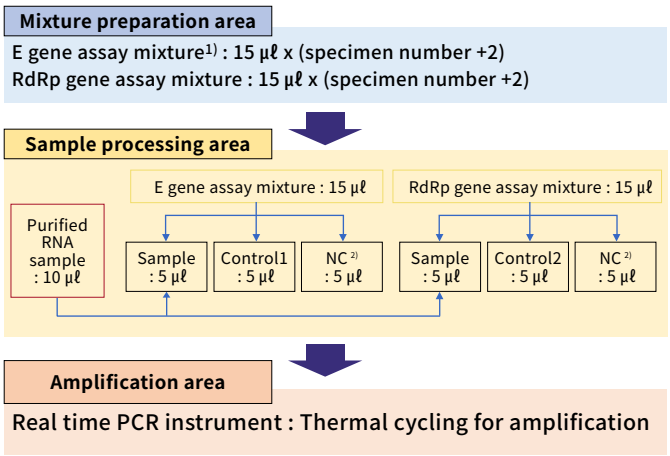
9.2 RNA extraction

* Validated Kit for extraction of nucleic acids
- QIAamp Viral RNA Mini Kit (QIAGEN, Cat no.52904)

RNA extraction should be performed using QIAamp Viral RNA Mini Kit (QIAgen) according to the manufacturer's instructions and using the following specimen, lysis buffer and elution volumes. Use RNA samples immediately or store at -70°C.

Extraction kit	Patient specimen	Lysis buffer	Elution volume
QIAamp Viral RNA Mini Kit	140 µℓ	560 µℓ	50 µℓ

Schematic Workflow



[1] E gene assay mixture contains E gene primer/probe set(s) and IPC primer/probe set(s)
[2] NC is negative control(DEPC DW) supplied by manufacturer
Control sets (Control 1 and NC for E gene assay, Control 2 and NC for RdRp gene assay) should be run with each batch

9.3 RT-qPCR preparation

① Mixture Preparation

*Mixture preparation should be performed in area designated for mixture preparation to avoid contamination.
Two aliquots of the nucleic acid extract are tested for each patient specimen, one for the E gene assay and one for the RdRp gene assay. Two assay mixtures are also prepared (E gene and RdRp gene).

i) Prepare E gene assay mixtures and RdRp gene assay mixtures in separate PCR tubes according to the following tables.

E gene assay mixture components	1 Reaction (Total volume : 15µℓ)	Volumes for N specimens (µℓ)
Master mix	10µℓ	10 x (N+2)
Primer Probe mix 1	1µℓ	1 x (N+2)
DEPC DW	4µℓ	4 x (N+2)

RdRp gene assay mixture components	1 Reaction (Total volume : 15µℓ)	Volumes for N specimens (µℓ)
Master mix	10µℓ	10 x (N+2)
Primer Probe mix 2	1µℓ	1 x (N+2)
DEPC DW	4µℓ	4 x (N+2)

ii) Pipette 15µℓ of each assay mixture into applicable wells.
Cover and transfer the plate into sample processing area.

② Sample Preparation

*Sample preparation should be performed at sample processing area
i) Add 5µℓ of the extracted RNA, control 1, control 2, and NC(DEPC DW) to the wells pre-filled with the assay mixtures.
ii) Seal the plate with sealing film and spin down the plate in a table top plate centrifuge.
iii) Insert the plate into the PCR instrument.

9.4 Software setting

For each PCR instrument and software, enter the following assay settings for the 1copy™ COVID-19 qPCR Multi Kit.

① Enter the reaction volume 20 µL and modify PCR conditions presented in the following table.

Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	60°C *	30 sec	

* Measure fluorescence at 60°C (FAM and Texas Red(or Red 610) channel)

② Select the type of measurement fluorescence as FAM and Texas Red(or Red 610).

※ Please refer to appendix 1. Software Setting for detailed instructions on how to set each instrument.

10. Quality Control

* Control 1, Control 2, and two negative controls(NC) (for E gene assay and RdRp gene assay) should be run with each batch.

- DEPC DW provided in kit is used as a negative control (NC). It is necessary to evaluate if any contamination of the reaction mix is present; it is evaluated in two wells of each test run, one for the E gene assay and one for the RdRp gene assay. This negative control is run through the entire test process, including extraction. If the volume of the NC reagent supplied with the kit is not sufficient, it would be acceptable for testing laboratories include a separate negative control (nuclease-free water). NC should be negative and not exhibit fluorescence growth curves that cross the threshold line. If a false positive occurs with NC reactions, sample contamination may have occurred. Invalidate the run and repeat the assay.
- Control 1 (E gene plasmid) and control 2 (RdRp gene plasmid) are used as positive controls. The positive controls are needed for assessment of amplification detection processes, primer and probe integrity and run validity.
  Each positive control should produce a positive result for the applicable target (Ct value ≤35). If expected positive reactivity is not achieved, the run should be invalidated and repeated with a new aliquot of control.
- IPC (Internal positive control) should be present in each clinical specimen, and is co-purified with target SARS-CoV-2 virus. Therefore, the IPC can be used as an extraction control and an internal control. The IPC should be detected in E gene reaction well. The IPC needs to be evaluated to determine whether the extraction and amplification procedure is valid or not. The IPC must be detected (Ct value ≤35) for a clinical specimen to be reported as negative for SARS-CoV-2 RNA.
  Failure to detect IPC in a clinical specimen may indicate improper extraction of nucleic acid resulting in loss of nucleic acid, carry-over of PCR inhibitors from clinical specimens, or absence of sufficient human cellular material in the specimen. If expected positive reactivity of the IPC is not achieved in a specimen that is negative for SARS-CoV-2, re-sampling and re-testing should be performed for that specimen.

Quality control requirements should be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

11. Interpretation of Results

11.1 Cut off value

For both Control 1, Control 2, IPC and clinical specimens, the cut off value for each applicable target to be considered "detected" (+) is a Ct value ≤ 35. An assay target is considered positive (detected) if there is a sigmoidal amplification curve with no higher value of Ct of 35 at threshold.

Ct value	Result
≤ 35	Detected (+)
> 35 or N/A	Not Detected (-)

Ct values above 35 for FAM and Texas Red(or Red 610) may be a result of unspecific amplification.

※ Set threshold values and baseline

Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor Gene-Q	LC 480	Begin	End
E gene (FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene (FAM/Green)	300	50,000	15,000	0.1		3	15
IPC (Texas Red/ Red610/ Orange)	100	20,000	5,000	0.1		3	15

※ Please refer to appendix 1. Software Setting for detailed instructions on how to set each instrument.

11.2 Interpretation, Controls

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. After the positive control, negative controls and IPC have been examined and determined to be valid and acceptable, assessment of clinical specimen test results should be performed. However, if a patient specimen detects a SARS-CoV-2 target, the result is valid regardless of whether the IPC is detected or not.

11.3 System suitability test - Interpretation of Control Results

Control 1 (E gene)	Control 2 (RdRp gene)	Negative control	Interpretation
+	+	-	Pass
-	+/-	+/-	Control Failure/ System suitability failed/ Retest*
+/-	-	+/-	
+/-	+/-	+	

* In the event of a control failure, specimen results should not be reported. Repeat the test run with new controls.

※ Note: Ct ≤35 = Detected (+), Ct>35 = Not Detected (-)

11.4 Patient specimen interpretation

E gene assay		RdRp gene assay Sample (FAM)	Interpretation
Sample (FAM)	IPC (Texas Red)		
+	+/-	+	Positive for SARS-CoV-2
-	+	-	Negative for SARS-CoV-2
+	+/-	-	Presumptive positive for SARS-CoV-2**
-	+/-	+	Positive for SARS-CoV-2
-	-	-	Invalid Result* / Repeat extraction and RT-PCR. If repeat result is invalid, consider collection of a new specimen.

* Invalid result due to potential sampling error or inhibition.

** Presumptive positive for SARS-CoV-2: A negative SARS-CoV-2 specific target result (RdRp gene) and a positive non-specific SARS-CoV-2 target result (E gene) may be suggestive of

1) a sample at concentrations near or below the limit of detection of the test,
2) a mutation in the RdRp target region in the oligo binding sites, or
3) infection with some other Sarbecovirus (e.g. SARS-CoV or some other Sarbecovirus previously unknown to infect humans), or
4) other factors. Sample should be retested.

For samples with a repeated presumptive positive result, 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.

※ Note: Ct ≤35 = Detected (+), Ct>35 = Not Detected (-)

12. Assay Limitations

Specimens 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 specimens must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.

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

False-positive results may arise from:
- Cross contamination during specimen handling or preparation
- Cross contamination between patient samples
- Specimen mix-up
- RNA contamination during product handling

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 indicates the detection of nucleic acid from SARS-CoV-2. Results do not reflect the viral load in the clinical specimens.

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.

Nasopharyngeal wash/aspirate or nasal aspirates and self-collected or healthcare provider collected nasal and mid-turbinate nasal swabs are additional acceptable upper respiratory specimens that can be tested with the 1copy™ COVID-19 qPCR Multi Kit; however, performance with these specimen types has not been determined.

This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal, anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens collected in a Universal Transport Medium (UTM) or Universal Viral Transport System (VTM). Testing of other sample types with the 1copy™ COVID-19 qPCR Multi Kit may result in inaccurate results.

As with any molecular test, mutations within the target regions of the 1copy™ COVID-19 qPCR Multi Kit could affect primer and/or probe binding resulting in failure to detect the presence of virus.

Based on the *in silico* analysis, SARS-coronavirus may cross-react with the 1copy™ COVID-19 qPCR Multi Kit. SARS-coronavirus is not known to be currently circulating in the human population, therefore is highly unlikely to be present in patient specimens.

13. Performance Evaluation

13.1 The Limit of Detection (LoD)

Studies were performed to determine the analytical limit of detection (LoD) of the 1copy™ COVID-19 qPCR Multi Kit. The LoD of the 1copy™ COVID-19 qPCR Multi Kit was established using one lot of reagents.

The RNA reference material for the experiment was AccuPlex™ SARS-CoV-2 Reference Material Kit (Seracare, Cat. No. 0505-0126, stock concentration 4226 copies/mL as determined by digital PCR). The reference material was serially diluted into pooled nasopharyngeal/oropharyngeal swab matrix.

The preliminary LoD was estimated using the Bio-Rad CFX96 instrument and included 5 sample replicates each of 5 dilution concentrations (100, 50, 25, 10, 5 copies/reaction). Confirmation of the final LoD for each instrument was performed with additional sample replicates tested at the same five concentrations but with 20 sample replicates tested at the three lowest concentrations, including the final LoD concentration.

The LoD is defined as the lowest concentration at which 19/20 replicates are positive for each assay target. The claimed LoD for the assay is 10 copies/reaction. The LoD for the 1copy™ COVID-19 qPCR Multi Kit is shown in the following table for each assay target and claimed PCR instrument.

Target	Bio-Rad CFX96	ABI 7500	ABI Quantstudio5	Light Cycler 480	Rotor Gene-Q
E gene	10copies/ reaction	10copies/ reaction	10copies/ reaction	10copies/ reaction	10copies/ reaction
RdRp gene	50copies/ reaction	50copies/ reaction	50copies/ reaction	50copies/ reaction	50copies/ reaction

* LoD Summary of the 1copy™ COVID-19 qPCR Multi Kit

PCR Instrument	Bio-Rad CFX96	ABI 7500	ABI Quantstudio5	Light Cycler 480	Rotor Gene-Q
LoD	10copies/ reaction	10copies/ reaction	10copies/ reaction	10copies/ reaction	10copies/ reaction

13.2 Clinical Evaluation

The performance of the 1copy™ COVID-19 qPCR Multi Kit test was evaluated using contrived clinical nasopharyngeal(NP) / Oropharyngeal(OP) swab specimens. Leftover individual NP/OP clinical swab specimens were determined to be negative for SARS-CoV-2 prior to inclusion in the study. Positive contrived specimens were prepared by spiking each individual clinical NP/OP swab specimen with RNA reference material, AccuPlex™ SARS-CoV-2 Reference Material Kit (Seracare, Cat. No. 0505-0126, stock concentration 4226 copies/mL as determined by digital PCR), at a concentration of approximately 2x LoD (20 samples), 3x LoD (5 samples), or 5x LoD (5 samples), respectively for each specimen type.

Negative NP/OP swab specimens were also tested in the study with 30 non-reactive specimens, respectively for each specimen type. All specimen identities were blinded and tested. Specimens were given a serial number by the researcher who conducted RNA spiking, and the specimen after spiking was recognized by the other experimenter by serial number(s) only. Study acceptance criteria for performance was defined as 95% agreement for specimens at 2x LoD, and 100% agreement for specimens at all other concentrations and for negative specimens.

The results showed 100% agreement with the expected results in the RNA spiked specimens and 100% agreement with the expected results in the negative specimens as shown in the following two tables.

* Nasopharyngeal swab specimens results

Target Concentration	Number concordant / Number Tested	Positive rate
2X LoD	20/20	100%
3X LoD	5/5	100%
5X LoD	5/5	100%
Negative	30/30	0%

* Oropharyngeal swab specimen results

Target Concentration	Number concordant / Number Tested	Positive rate
2X LoD	20/20	100%
3X LoD	5/5	100%
5X LoD	5/5	100%
Negative	30/30	0%

13.3 Inclusivity

The inclusivity of 1copy™ COVID-19 qPCR Multi Kit was evaluated using *in silico* analysis of the assay primers and probes in relation to 4254 SARS-CoV-2 sequences available in the GISAID gene database on May 17, 2020 for two targets, E and RdRp.

For the E target, 1copy™ COVID-19 qPCR Multi Kit had 100% match to all sequences with the exception of 4 sequences that had a single mismatch. For the RdRp target, 1copy™ COVID-19 qPCR Multi Kit had 100% match to all sequences with the exception of 6 sequences that had a single mismatch. None of these mismatches found for either targets are predicted to have a negative impact on the performance of the assay, given the location of the mismatches in the primer and probe regions respectively for the five variants. These mismatches are not predicted to adversely affect probe and primer binding or reduce assay efficiency.

13.4 Cross-reactivity

List of Organisms analyzed using *in silico* analysis

Other high priority pathogens from the same genetic family as SARS-CoV-2	Other organisms that may be present in respiratory specimens
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius

An *in silico* analysis for possible cross-reactions with all the organisms listed in the Table above was conducted by mapping primers and probes for both E gene and RdRp primers and probes in the 1copy™ COVID-19 qPCR Multi Kit individually to the sequences downloaded from the NCBI database. Potential cross-reaction is possible if there is >80% homology between the database sequence and the target primers/probes of the assay. Results from the analysis showed that the RdRp primers and probe are specific for SARS-CoV-2 and E primers and probe are specific for SARS-CoV-2 and SARS-coronavirus.

Cross reactivity is not expected with other organisms listed in table above based on the *in silico* analysis.
To further evaluate the potential for cross-reactivity of the 1copy™ COVID-19 qPCR Multi Kit target sequences, wet-testing was performed for selected microorganisms and viruses that may be present in respiratory specimens. For cross-reactivity test, synthetic RNA of SARS-CoV-2 specific E gene and RdRp gene were separately evaluated for potential-cross-reactivity. All samples prepared with these synthetic RNA sequences were positive for the expected corresponding primer/probe mixture only. Testing also included respiratory viral pathogens (Influenza A virus (H3N2), Influenza A virus (H1N1)), Parainfluenza virus 1, Parainfluenza virus 2, Rhinovirus 14, Enterovirus 71), as well as *Escherichia coli* and human total RNA.

Samples were prepared at high microorganism concentrations as shown in the following table. A total of five replicates were tested for each potential cross-reactant. No unexpected cross-reactivity was observed for the organisms and viruses listed. The results can be seen in the table below.

Wet-testing cross-reactivity of the 1copy™ COVID-19 qPCR Multi Kit

Organism	Concentration	Results E Gene (#detected/tested)	Results RdRp Gene (#detected/tested)
Synthetic RNA of COVID-19 specific RdRp gene	5 x 10^2 copies/mL	Not detected (0/5)	Detected (5/5)
Synthetic RNA of beta-coronavirus specific E gene	5 x 10^2 copies/mL	Detected (5/5)	Not Detected (0/5)
Influenza A virus (H3N2) (Ref. KBPV_VR_32)	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)
Influenza A virus (H1N1) (Ref. KBPV_VR_33)	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)
Parainfluenza virus 1	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)
Parainfluenza virus 2	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)
Rhinovirus 14	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)
Enterovirus 71	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)
Escherichia coli	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)
Human total RNA	5 x 10^7 copies/mL	Not detected (0/5)	Not detected (0/5)

14. References

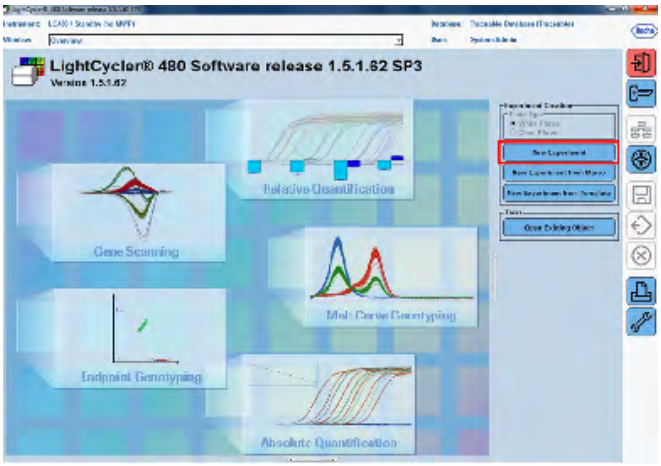
1. Centers for Disease Control and Prevention. https://www.cdc.gov/coronavirus/2019ncov/index.html. Accessed February 9, 2020.
2. Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). http://www.cdc.gov/biosafety/publications/
3. Clinical and Laboratory Standards Institute. Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline. Document M29 (refer to latest edition).
4. Clinical and Laboratory Standards Institute. Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline. CLSI Document MM13-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
5. World Health Organization. Laboratory Biosafety Manual. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.
6. World Health Organization. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans. 3. Molecular assays to diagnose 2019-nCoV. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2
7. WHO interim guidance for laboratory testing for 2019 novel coronavirus (2019-nCoV) in humans; 19 March 2020. https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117

15. Glossary of Symbols

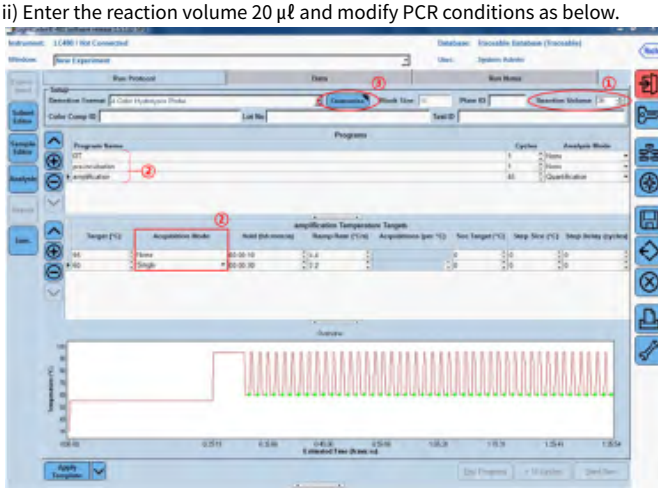
REF	Catalog Number
CE	This product fulfills the requirement for directive on in vitro diagnostic medical devices (Conformite Europeenne)
IVD	In-Vitro-Diagnostic Medical Device
EC REP	Authorized representative in the European community
LOT	Batch Code
	Contains sufficient for tests
	Use By Date
	Manufacturer
	Consult Instructions for use
	Temperature limitation
	Caution

Appendix 1. Software Setting

① Light Cycler 480
(Roche, Product No. 05015278001)
i) Run software and click “New Experiment”



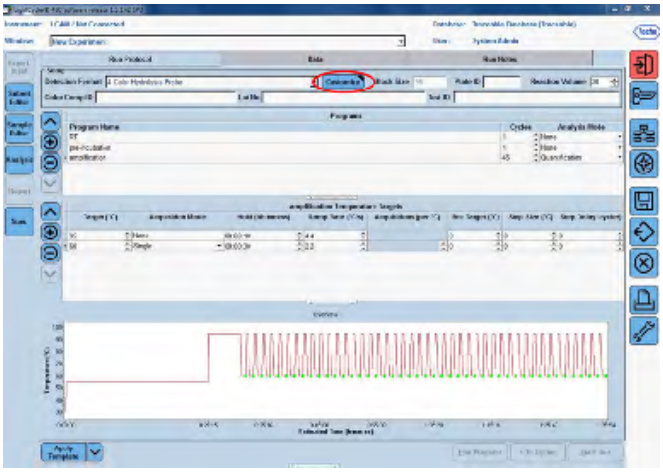
ii) Enter the reaction volume 20 µℓ and modify PCR conditions as below.

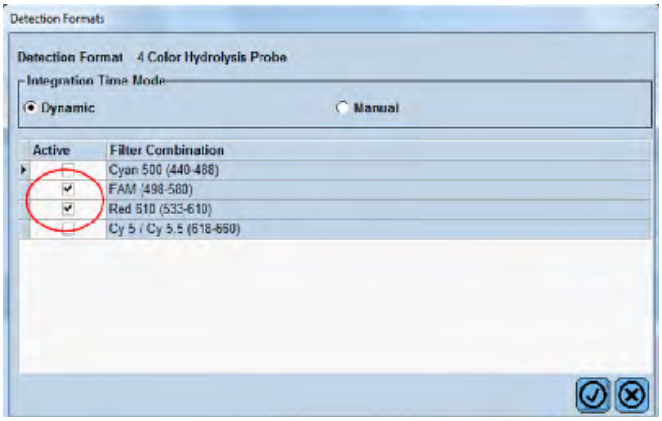


Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	60°C *	30 sec	

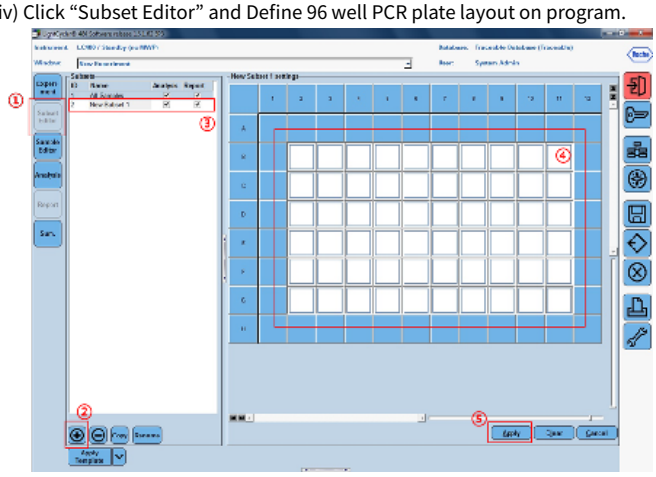
* Measure florescence at 60°C (FAM and Red 610) channel

iii) Click “Customize” and select “FAM” & “Red 610”.

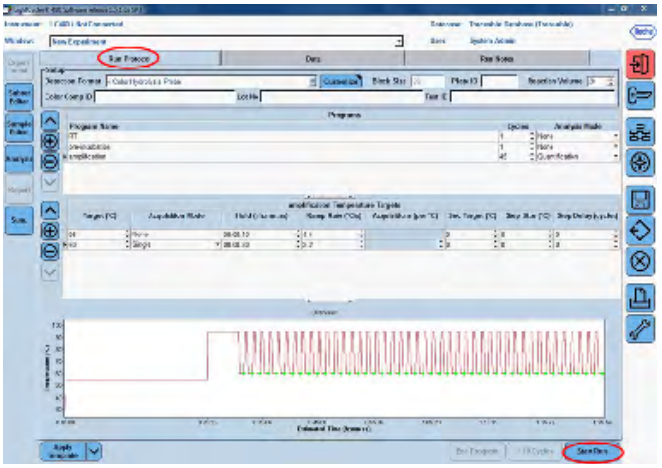




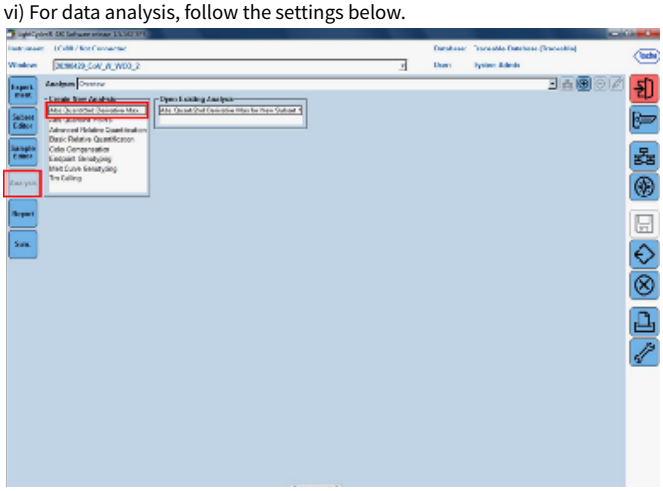
iv) Click “Subset Editor” and Define 96 well PCR plate layout on program.



v) Click “Run Protocol” on menu bar above and then “Start Run”



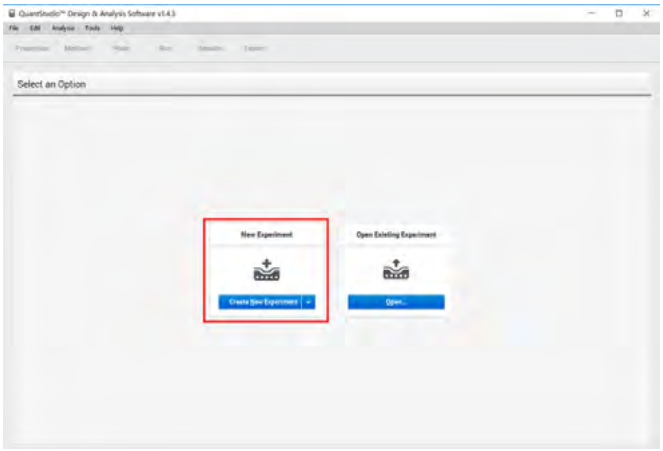
vi) For data analysis, follow the settings below.



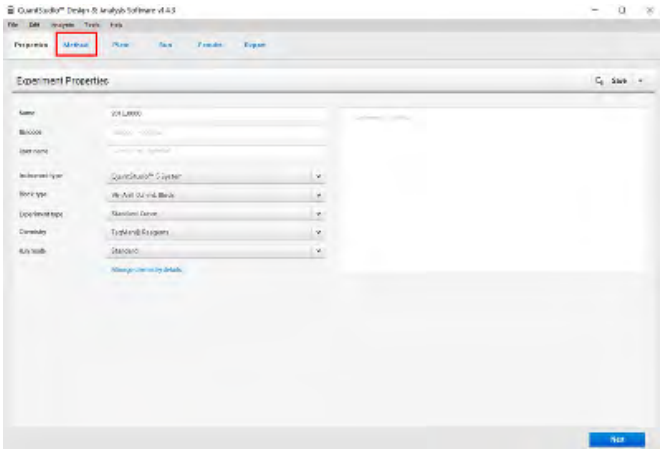
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene (FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene (FAM/Green)	300	50,000	15,000	0.1		3	15
IPC (Texas Red/ Red 610/ Orange)	100	20,000	5,000	0.1		3	15

② Applied Biosystems Quantstudio5
(Thermo Fisher Scientific, Product No. A28134)

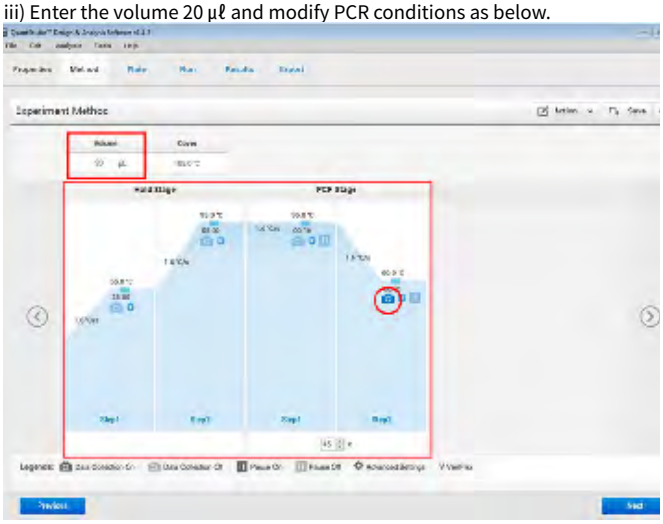
i) Run software and click “Create New Experiment” of “New Experiment”



ii) Click “Method” on the menu bar above.



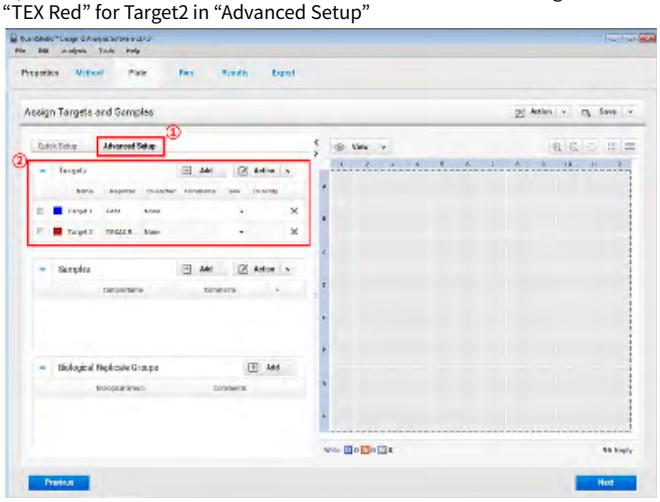
iii) Enter the volume 20 μℓ and modify PCR conditions as below.



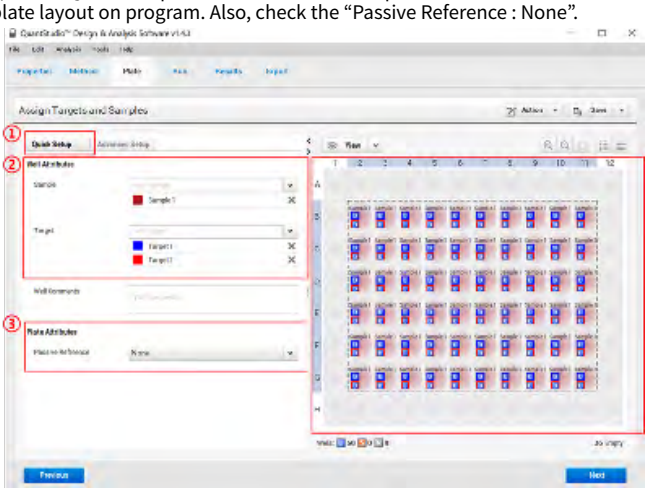
Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	60°C *	30 sec	

* Measure florescence at 60°C (FAM and Red 610) channel

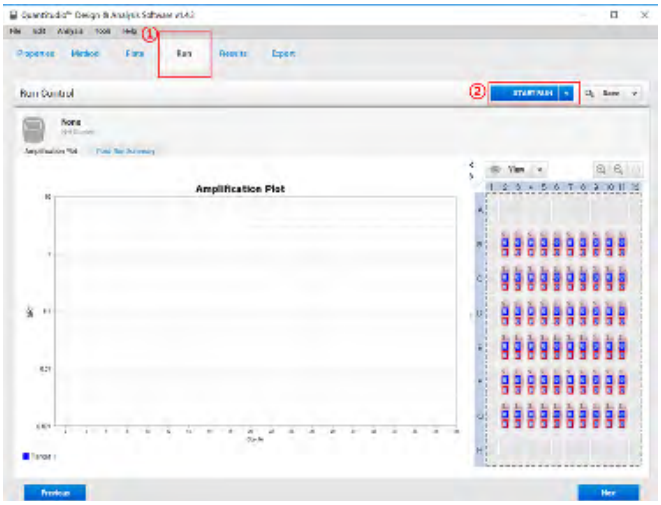
iv) Click “Plate” on the menu bar above and select “FAM” for Target1 and “TEX Red” for Target2 in “Advanced Setup”



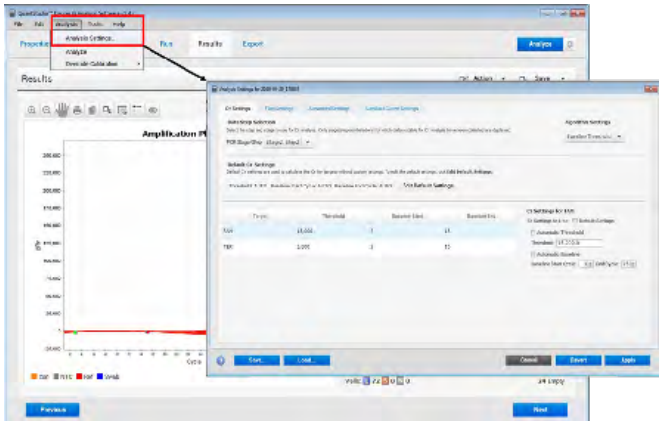
v) Click “Quick Setup” next to “Advanced Setup” and define 96 well PCR plate layout on program. Also, check the “Passive Reference : None”.



vi) Click “Run ” on the menu bar above and then “Start Run”



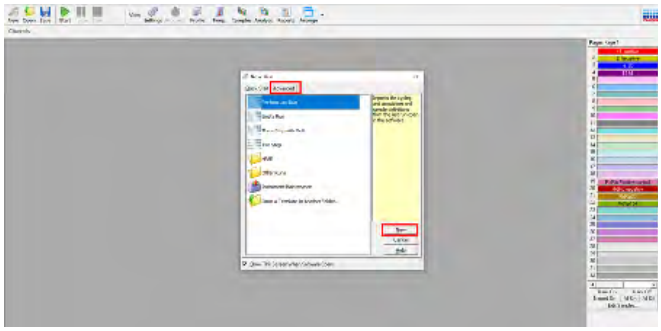
vii) For data analysis, follow the settings below.



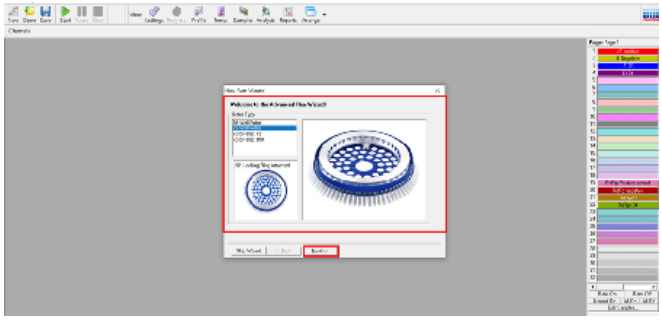
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene (FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene (FAM/Green)	300	50,000	15,000	0.1		3	15
IPC (Texas Red/ Red 610/ Orange)	100	20,000	5,000	0.1		3	15

③ Rotor-Gene Q 5plex HRM (Qiagen, Product No. 9001580)

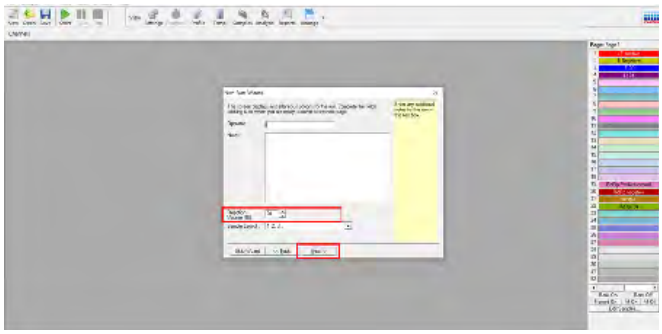
i) Run software and click “Advanced” and click “New”.



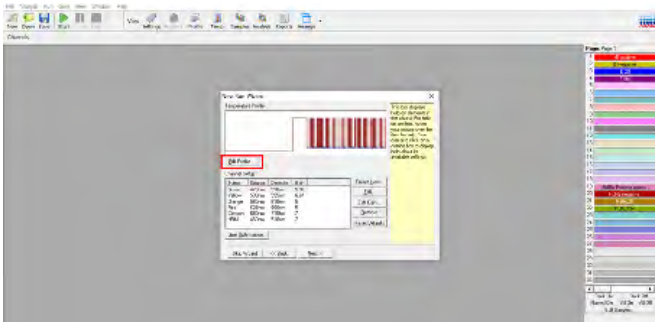
ii) Check the Rotor type and Click “Next”.



iii) Enter the reaction volume 20 µℓ and click “Next”.



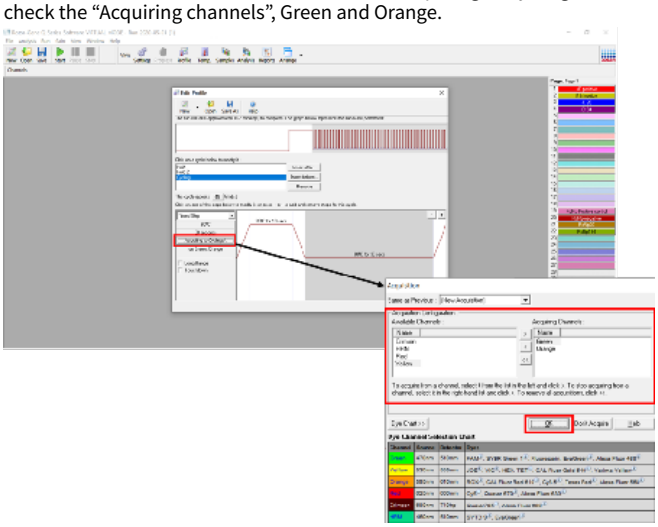
iv) Click “Edit profile” and modify PCR conditions as below.



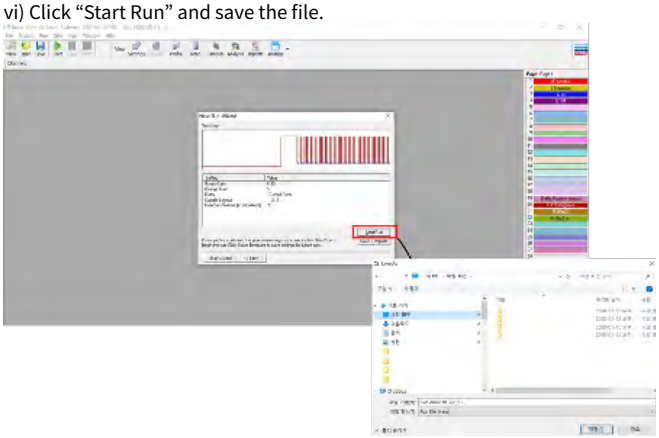
Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	60°C *	30 sec	

* Measure florescence at 60°C (Green and Orange) channel

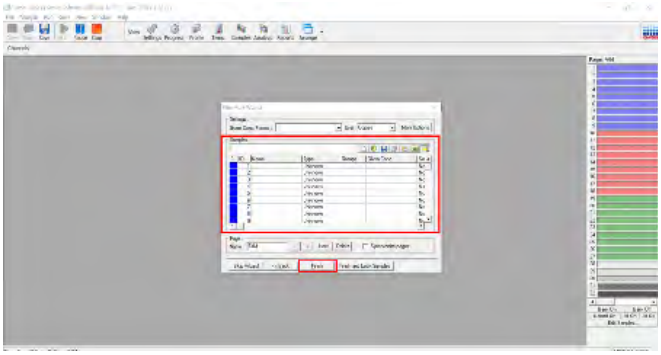
v) To measure fluorescence at 60°C, click the “Acquiring to Cycling A” and check the “Acquiring channels”, Green and Orange.



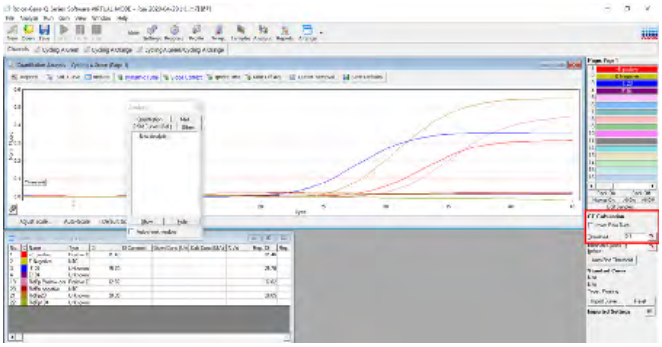
vi) Click “Start Run” and save the file.



vii) Define the samples and click “Finish”.



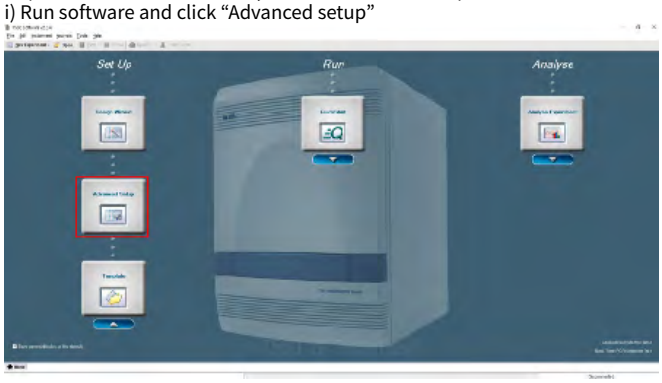
viii) For data analysis, follow the settings below.



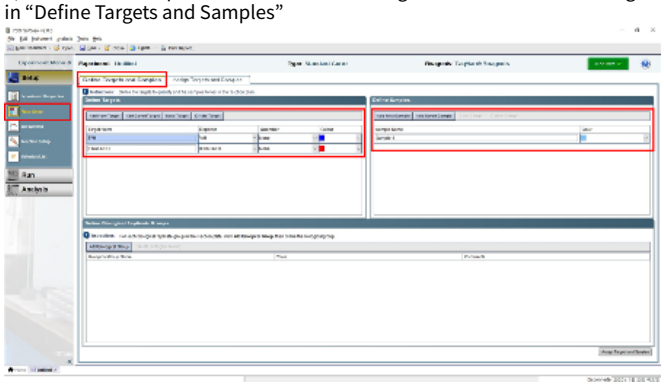
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene (FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene (FAM/Green)	300	50,000	15,000	0.1		3	15
IPC (Texas Red/ Red 610/ Orange)	100	20,000	5,000	0.1		3	15

④ Applied Biosystems 7500 Real-Time PCR Instrument system (Thermo Fisher Scientific, Product No. 4345241)

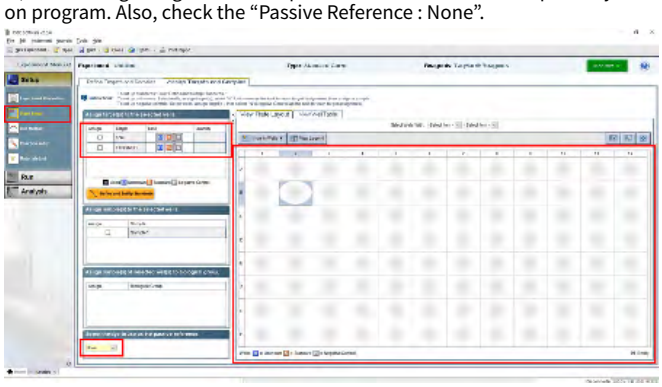
i) Run software and click “Advanced setup”



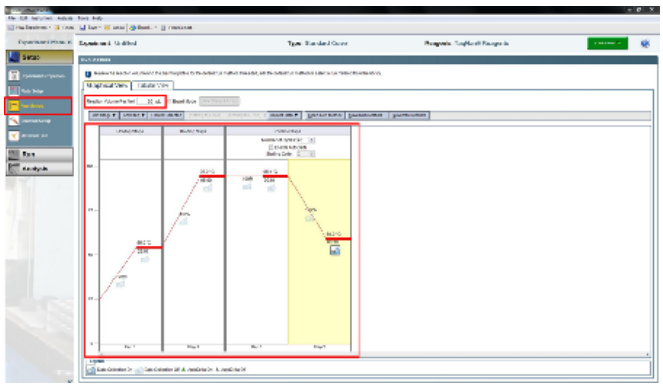
ii) Click “Plate setup” and select “FAM” for Target1 and “TEX Red” for Target2 in “Define Targets and Samples”



iii) Click “Assign Targets and Samples” and define 96 well PCR plate layout on program. Also, check the “Passive Reference : None”.



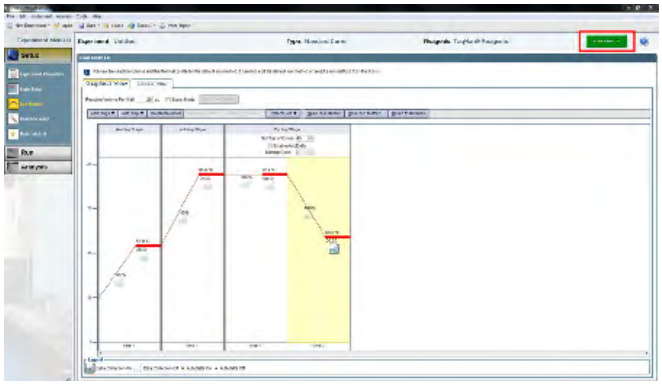
iv) Click “Run Method” and enter the volume 20 µℓ and modify PCR conditions as below.



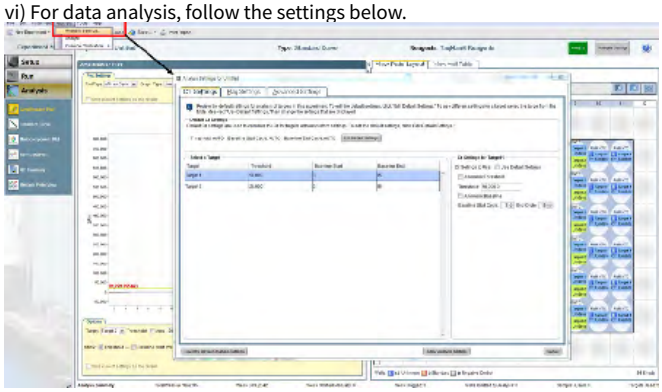
Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	60°C *	30 sec	

* Measure florescence at 60°C (FAM and TexRED) channel

v) Click “Start Run”.



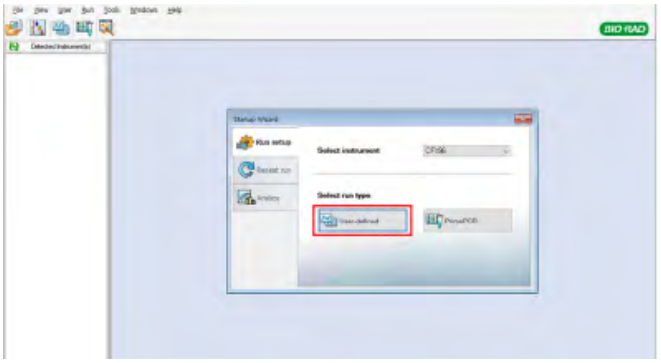
vi) For data analysis, follow the settings below.



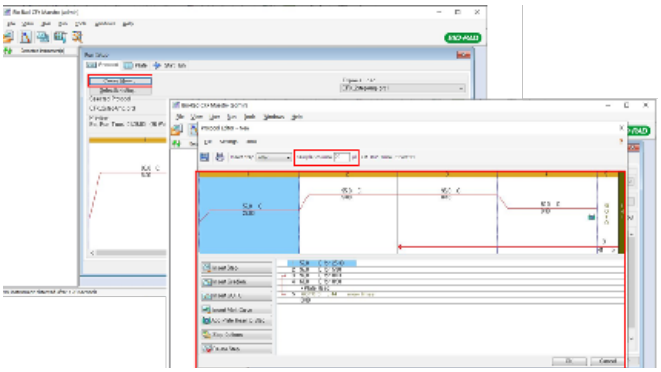
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene (FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene (FAM/Green)	300	50,000	15,000	0.1		3	15
IPC (Texas Red/ Red 610/ Orange)	100	20,000	5,000	0.1		3	15

⑤ CFX96™ Real-Time PCR Detection system (BIO-RAD, Product No. 1854095-IVD)

i) Run software and click “User-defined”



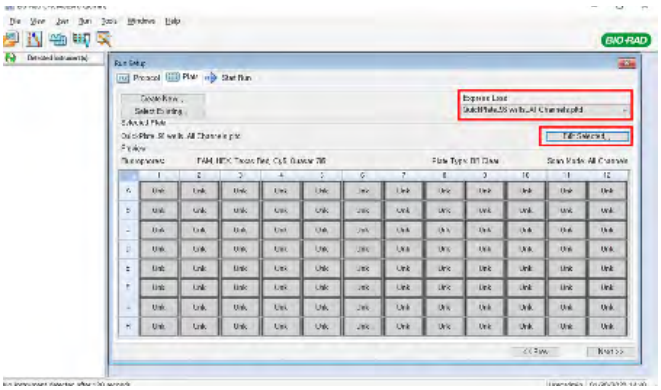
ii) Click “Create New” and enter the volume 20 µℓ and modify PCR conditions as below.



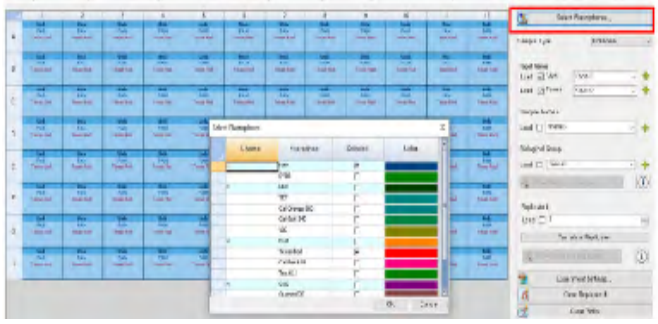
Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	60°C *	30 sec	

* Measure florescence at 60°C (FAM and TexRED) channel

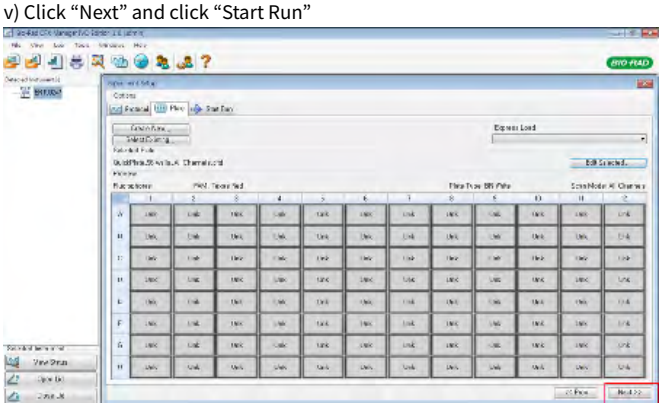
iii) Click “Plate” and check the “Express Load : QuickPlate_96 wells_All Channels.pltd” and click “Edit selected”.

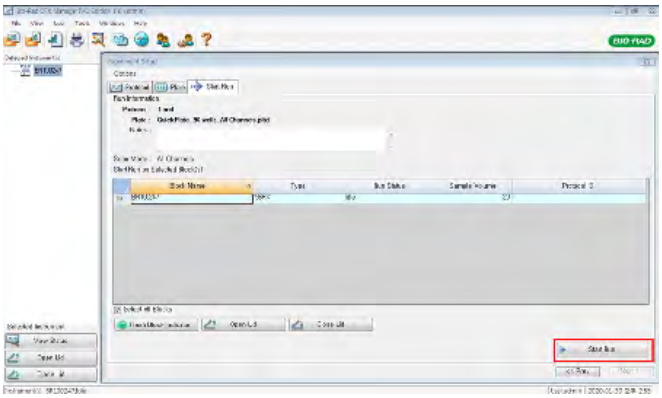


iv) Click “Select Fluorophores” and check FAM and Texas Red. Also, Define 96 well PCR plate layout on program.

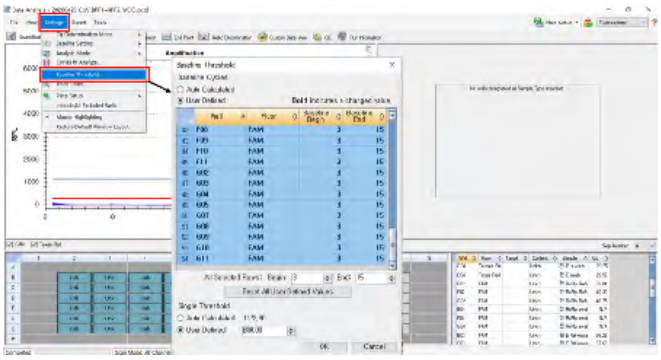


v) Click “Next” and click “Start Run”





vi) For data analysis, follow the settings below.



Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene (FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene (FAM/Green)	300	50,000	15,000	0.1		3	15
IPC (Texas Red/ Red 610/ Orange)	100	20,000	5,000	0.1		3	15

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