1copy™ COVID-19 qPCR Multi Kit

(Cat no. M22MD100M)

Instructions for Use

For *in vitro* diagnostic use

For Emergency Use Authorization Only

Prescription Use Only

1drop Inc.
A-203, Keumkang Penterium IT Tower 215, Galmachi-ro, Jungwon-gu,
Seongnam-si, Gyeonggi-do, 13217, REPUBLIC OF KOREA
TEL: +82 31 747 0109
FAX : +82 70 4275 1248
Email: cs@1drop.co.kr
Website: www.1drop.co.kr

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1. Description

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

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.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all test 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 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. 1copy™ COVID-19 qPCR Multi Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

3. Principle of the Assay

The 1copy™ COVID-19 qPCR Multi Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed according to "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 midturbinate swabs, nasopharyngeal wash/aspirates and nasal aspirate specimens) are extracted using QIAamp Viral RNA Mini Kit RNA mini kit (QIAgen). After extraction, the purified nucleic acid is first reverse-transcribed into cDNA by reverse transcriptase, and then subsequently amplified by Taq DNA polymerase in the rRT-PCR instrument. In the PCR amplification, the 5' nuclease activity of Taq DNA polymerase causes the degradation of the TaqMan probe, causing 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 (human GAPDH gene) in E gene assay mixture (first well). The kit uses dUTP and UNG enzymes to prevent contamination of amplification products.

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

4. Kit Contents (Materials Provided)

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

※ 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 (1~10㎕, 10~100㎕, 100~1000㎕)

· 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

· Federal Law restricts this device to sale by or on the order of a licensed practitioner.

· 1copy™ COVID-19 qPCR Multi Kit is for *in vitro* diagnostic use only.

· For use under Emergency Use Authorization Only.

This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.

- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

· 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 using safe laboratory procedures. Refer to 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 package insert carefully prior to operation 1copy™ COVID-19 qPCR Multi Kit is only for emergency use with a prescription, as an *in vitro* diagnostic test. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.

· False positive and false negative results can be caused by poor specimen quality, improper specimen collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation 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 in 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 kit lots.

· This product is intended for professional use only and should be used 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 mix and spin down.

· Prepare mixture 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 with the 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 discarded 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 review of various results such as the patient’s symptoms.

· Even if the test results of this product are ‘negative’, it should be interpreted by an experienced specialist and review of various results such as the patient’s symptoms without excluding infection.

8. Reagent Storage and Handling

· Store the kit below -20°C.

· Expiration date for kit is indicated on the packing box.

· 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 to be tested can be stored for 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

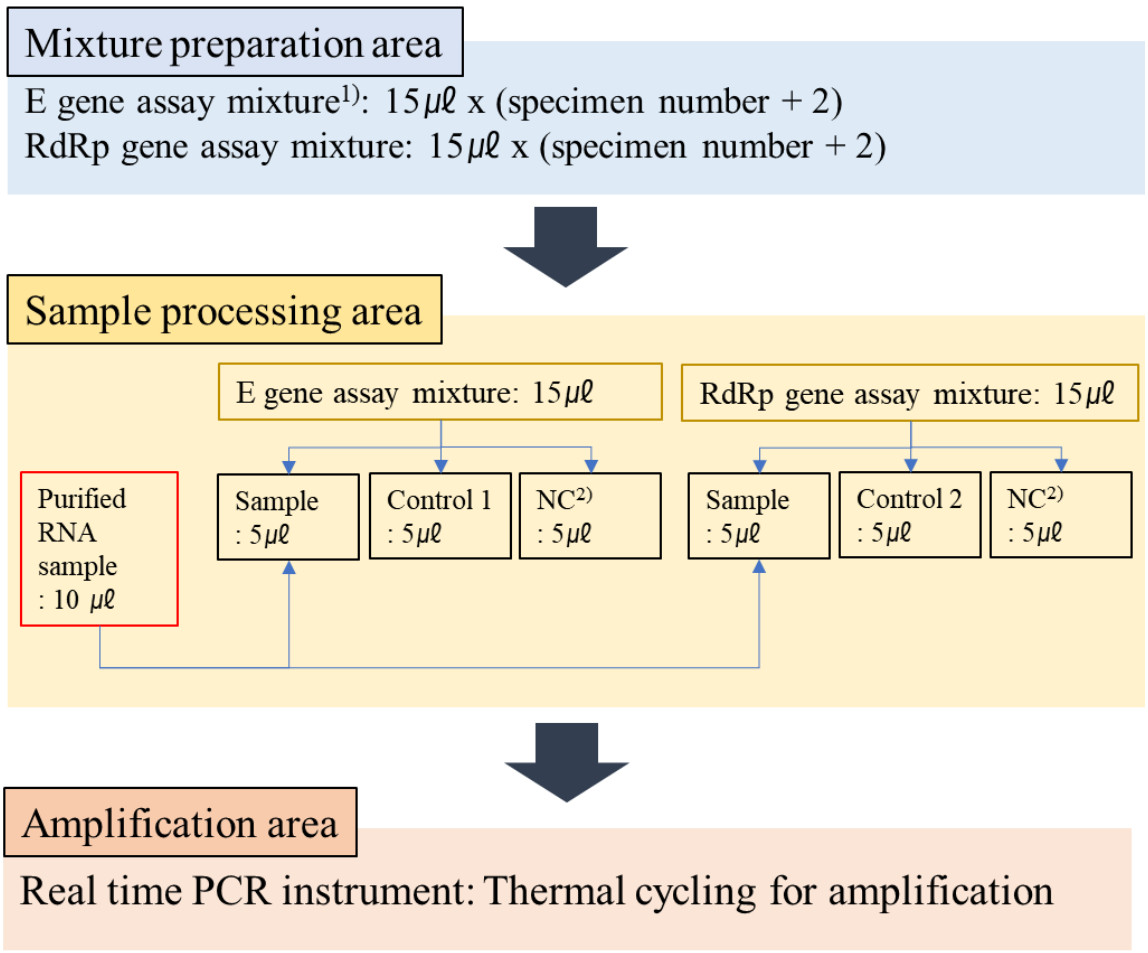
* Validated Kit for extraction of nucleic acids

- 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 ㎕	560 ㎕	50 ㎕

Schematic Workflow



[1] E gene assay mixture contains E gene primer/probe set(s) and GAPDH primer/probe set(s) for IPC detection
[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 at mixture preparation area 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).

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

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

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

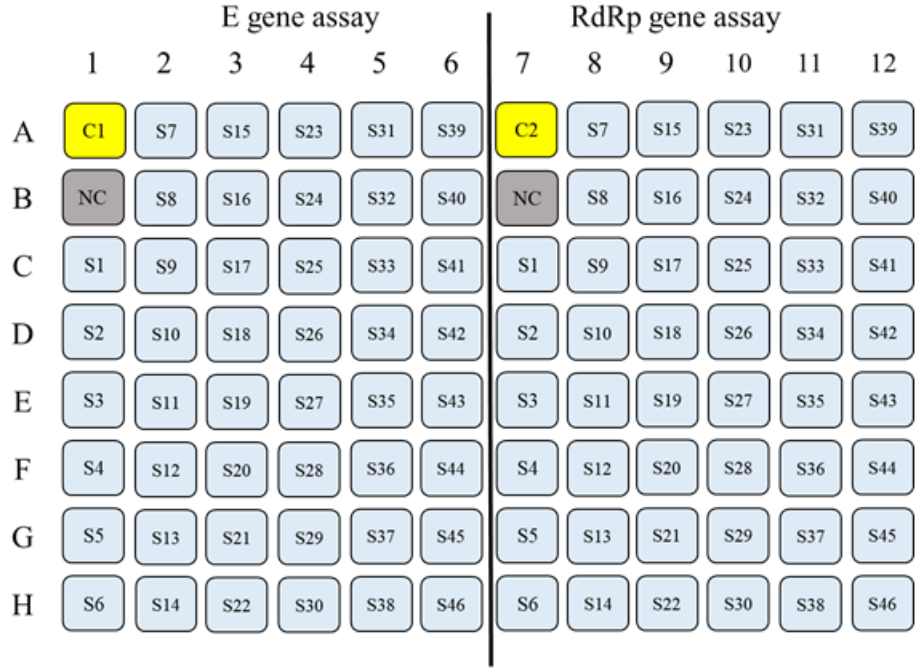
ii) Pipette 15㎕ of each assay mixture into applicable wells according to the plate layout below. Cover and transfer the plate into sample processing area.

② Sample Preparation

*Sample preparation should be performed at sample processing area

i) Add 5㎕ of the extracted RNA, control 1, control 2, and NC(DEPC DW) to the wells pre-filled with the assay mixtures according to plate layout below.

ii) Plate layout is as follows (example).



- C1: Control 1 (E gene positive control)
- C2: Control 2 (RdRp gene positive control)
- NC: Negative control (DEPC DW)
- S: Patient RNA sample

iii) Seal the plate with sealing film and spin down the plate in a table top plate centrifuge.

iv) Insert the plate into the PCR instrument.

9.4 Software setting

* The 1copy™ COVID-19 qPCR Multi Kit has been validated with the following Real-Time PCR instruments and software:

· 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)

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 reaction conditions presented in the following table.

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

* Measure fluorescence at 60℃ (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 the Appendix 1 for detailed instructions on how to use each instrument.

10. Quality Control

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

· DEPC DW provided in kit is used as a negative control (NC). It is needed to evaluate if any contamination of the reaction mix and is evaluated in two wells of each test run, one for the E gene assay and one for the RdRp 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 that 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 and with control having a target concentration of ~1,000 copies/mL. The positive controls are needed for assessment of amplification and detection processes as well as primer and probe integrity and to evaluate run validity.
Each positive control should produce a positive result for the applicable target (Ct value ≤40 Ct). If expected positive reactivity is not achieved, the run should be invalidated and repeated with a new aliquot of control.

· IPC (Internal positive control, endogenous human GAPDH mRNA) 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 is needed to evaluate, whether the extraction and amplification procedure is valid or not. The IPC must be detected (Ct ≤40) for a clinical specimen to be reported as negative for SAR-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 SAR-CoV-2, re-sampling and re-testing should be performed for the 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 cutoff value for each applicable target to be considered detected (+) is a Ct value of ≤40. An assay target is considered positive (detected) if there is a sigmoidal amplification curve with no higher than Ct of 40 at threshold value.

Ct value	Result
≤ 40	Detected (+)
> 40 or N/A	Not Detected (-)

Ct values above 40 for FAM and Texas Red(or Red 610) signals may be the result of unspecific amplification.

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.

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 ≤40 = Detected (+), Ct>40 = Not Detected (-)

11.4 Patient specimen interpretation

E gene assay		RdRp gene assay	Interpretation
Sample (FAM)	**IPC (Texas Red)**	**Sample (FAM)**	
+	+/-	+	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 target) 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 ≤40 = Detected (+), Ct>40 = 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 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
- Using unauthorized extraction or assay reagents
- The presence of RT-PCR inhibitors
- Mutation in the SARS-CoV-2 virus o 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 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.

The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

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.

The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

13. Conditions of Authorization for Laboratory

The 1copy™ COVID-19 qPCR Multi Kit assay's Letter of Authorization, User Manual, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the 1copy™ COVID-19 qPCR Multi Kit ("your product" in the conditions below), the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

b) Authorized laboratories using your product will use your product as outlined in the Instructions for Use only. Deviation from the authorized procedures, such as the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

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

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

e) Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and 1drop Inc. (sales@1drop.co.kr, +82 31 747 0109) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which you become aware.

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

g) 1drop Inc., its authorized distributor(s) and authorized laboratories using the 1copy™ COVID-19 qPCR Multi 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."

14. Performance Evaluation

14.1 The Limit of Detection (LoD)

Studies were performed to determine the analytical limit of detection (LoD) of the 1copy™ COVID-19 Multi qPCR Kit. The LoD of the 1copy™ COVID-19 Multi qPCR 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 (500, 400, 300, 200, 100 copies/mL). 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 200 copies/mL. Preliminary testing and confirmatory study results are presented in the following tables.

Preliminary LoD Range-Finding Results (Bio-Rad CFX96)

Concentration RNA copies/mL	Assay 1 (E gene)			Assay 2 (RdRp gene)		
	Mean Ct	%CV	Detection rate	Mean Ct	%CV	Detection rate
500	37.87	0.86	5/5	38.58	0.87	5/5
400	38.23	0.86	5/5	38.78	0.45	5/5
300	38.56	0.66	5/5	39.15	0.82	5/5
200	38.62	0.85	5/5	39.09	0.68	5/5
100	38.94	0.51	4/5	39.57	0.90	4/5

Confirmatory LoD Testing, Bio-Rad CFX96

Concentration (copies/mL)	Detection rate		Mean Ct and %CV					
	E gene	RdRp gene	E gene		IPC		RdRp gene	
			Mean Ct	%CV	Mean Ct	%CV	Mean Ct	%CV
500	100% (5/5)	100% (5/5)	37.59	1.07	26.89	2.99	37.88	0.47
400	100% (5/5)	100% (5/5)	38.13	1.13	27.68	6.85	38.23	0.92
300	100% (20/20)	100% (20/20)	38.7	0.89	27.52	5.30	38.74	1.25

200	100% (20/20)	100% (20/20)	38.91	1.61	27.26	4.94	39.00	1.61
100	85% (17/20)	80% (16/20)	39.23	1.11	27.09	5.49	39.16	0.95

Confirmatory LoD Testing, ABI 7500

Concentration (copies/mL)	**Detection rate**		**Mean Ct and %CV**					
	E gene	**RdRp gene**	**E gene**		**IPC**		**RdRp gene**	
			Mean Ct	%CV	Mean Ct	%CV	Mean Ct	%CV
500	100% (5/5)	100% (5/5)	37.32	0.69	28.09	5.51	37.50	0.62
400	100% (5/5)	100% (5/5)	37.63	1.50	27.35	5.54	37.71	1.94
300	100% (20/20)	100% (20/20)	38.01	1.80	27.71	5.22	38.12	1.30
200	100% (20/20)	100% (20/20)	38.75	1.46	27.48	5.57	38.52	1.17
100	75% (15/20)	80% (16/20)	39.24	1.00	27.19	5.99	39.07	1.49

Confirmatory LoD Testing, ABI Quantstudio5

Concentration (copies/mL)	**Detection rate**		**Mean Ct and %CV**					
	E gene	**RdRp gene**	**E gene**		**IPC**		**RdRp gene**	
			Mean Ct	%CV	Mean Ct	%CV	Mean Ct	%CV
500	100% (5/5)	100% (5/5)	36.91	1.28	26.93	5.68	37.41	1.10
400	100% (5/5)	100% (5/5)	37.30	0.28	27.59	4.39	37.83	0.24
300	100% (20/20)	100% (20/20)	37.95	1.32	26.52	5.70	38.20	1.06
200	100% (20/20)	100% (20/20)	38.32	1.85	26.58	5.59	38.61	1.21
100	80% (16/20)	85% (17/20)	39.05	1.87	26.61	5.59	39.05	1.58

Confirmatory LoD Testing, Roche Light Cycler 480

Concentration (copies/mL)	**Detection rate**		**Mean Ct and %CV**					
	E gene	**RdRp gene**	**E gene**		**IPC**		**RdRp gene**	
			Mean Ct	%CV	Mean Ct	%CV	Mean Ct	%CV
500	100% (5/5)	100% (5/5)	36.64	1.09	27.35	6.87	36.67	1.00
400	100% (5/5)	100% (5/5)	37.08	0.93	26.73	6.08	37.02	1.21
300	100% (20/20)	100% (20/20)	37.60	0.77	26.34	6.15	37.40	0.93

200	100% (20/20)	100% (20/20)	38.12	1.56	25.99	5.14	37.94	1.26
100	90% (18/20)	80% (16/20)	38.58	2.41	26.14	6.25	38.83	1.98

Confirmatory LoD Testing, QIAGEN Rotor Gene-Q

Concentration (copies/mL)	**Detection rate**		**Mean Ct and %CV**					
	E gene	**RdRp gene**	**E gene**		**IPC**		**RdRp gene**	
			Mean Ct	%CV	Mean Ct	%CV	Mean Ct	%CV
500	100% (5/5)	100% (5/5)	37.85	1.15	28.31	6.68	37.83	1.53
400	100% (5/5)	100% (5/5)	38.43	0.88	28.53	3.88	38.32	1.58
300	100% (20/20)	100% (20/20)	38.79	1.50	28.35	5.56	38.74	0.92
200	100% (20/20)	100% (20/20)	39.28	1.05	28.46	5.95	39.22	1.71
100	90% (18/20)	80% (16/20)	39.35	0.75	28.91	4.84	39.35	1.32

The final LoD for the 1copy™ COVID-19 qPCR Multi Kit is shown in the following table for each assay target and claimed PCR instrument.

LoD	Bio-Rad CFX96	ABI 7500	ABI Quantstudio5	Light Cycler 480	Rotor gene-Q
RdRp gene	200copies/㎖	200copies/㎖	200copies/㎖	200copies/㎖	200copies/㎖
E gene	200copies/㎖	200copies/㎖	200copies/㎖	200copies/㎖	200copies/㎖

14.2 Clinical Evaluation

Clinical evaluation was conducted in accordance with the revised “Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency” on May 11, 2020. Clinical evaluation of the 1copy™ COVID-19 qPCR Multi Kit was evaluated using clinical nasopharyngeal and oropharyngeal swab specimens diagnosed with the FDA-authorized real-time RT-PCR assay. 30 positive and 30 negative samples were used for each nasopharyngeal swab and oropharyngeal swab samples (A total of 120 samples were used). All samples were tested in randomized and blinded fashion. The extraction of RNA from each kit was carried out in accordance with the IFU of each manufacturer. Both kits extracted RNA using QIAamp Viral RNA Mini Kit (QIAGEN, Cat no.52904). The results are determined by the positive and negative percentage agreement of each sample and the acceptance criteria are 95%.

* Nasopharyngeal swab specimens results

		FDA-authorized real-time RT-PCR assay		
		Positive	Negative	Total
1copy™ COVID-19 qPCR Multi Kit	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive percent agreement		100% (95%CI: 92.50% ~ 100%)		
Negative percent agreement		100% (95%CI: 92.50% ~ 100%)		

* Oropharyngeal swab specimen results

		FDA-authorized real-time RT-PCR assay		
		Positive	Negative	Total
1copy™ COVID-19 qPCR Multi Kit	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive percent agreement		100% (95%CI: 92.50% ~ 100%)		
Negative percent agreement		100% (95%CI: 92.50% ~ 100%)		

14.3 Inclusivity

The inclusivity of 1copy™ COVID-19 Multi qPCR Kit was evaluated using *in silico* analysis of the assay primers and probes in relation to 108,729 SARS-CoV-2 sequences available in the GISAID gene database from 31 August 2021 to 5 September 2021 for two targets, E and RdRp.

For the E target, 1copy™ COVID-19 Multi qPCR Kit had 100% match to all sequences with the exception of 358 sequences that had a single mismatch. For the RdRp target, 1copy™ COVID-19 Multi qPCR Kit had 100% match to all sequences with the exception of 579 sequences that had a single mismatch. None of these mismatches found for both 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.

1copy™ COVID-19 qPCR Multi Kit (1drop Inc., Cat. No. M22MD100T)					
	Number of Analyzed sequences	**Number of Confirmed 100% homology sequences**	**Number of Single Mutation sequences**	**Number of Above Single Mutation sequences**	**Number of Exclusive sequences ***
RdRp gene Primer Forward	108,729	108,296 (99.602%)	427 (0.393%)	0	6 (0.006%)
RdRp gene Primer Reverse	108,729	108,703 (99.976%)	26 (0.024%)	0	0 (0%)
RdRp gene Probe	108,729	108,601 (99.882%)	126 (0.116%)	0	2 (0.002%)

E gene Primer Forward	108,729	108,576 (99.859%)	140 (0.129%)	0	13 (0.012%)
E gene Primer Reverse	108,729	108,572 (99.856%)	127 (0.117%)	0	30 (0.028%)
E gene Probe	108,729	108,607 (99.888%)	91 (0.084%)	0	31 (0.029%)

14.4 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 Mulit 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)

14.5 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 QIAamp Viral RNA Mini Kit (QIAGEN, Cat no.52904) and CFX96™ Real-time PCR Detection System (Bio-Rad, Product No. 1854095-IVD). The results are summarized in the following Table.

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

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal swab	1.8x10^4 NDU/mL	N/A
MERS-CoV		N/A	ND

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

15. 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.

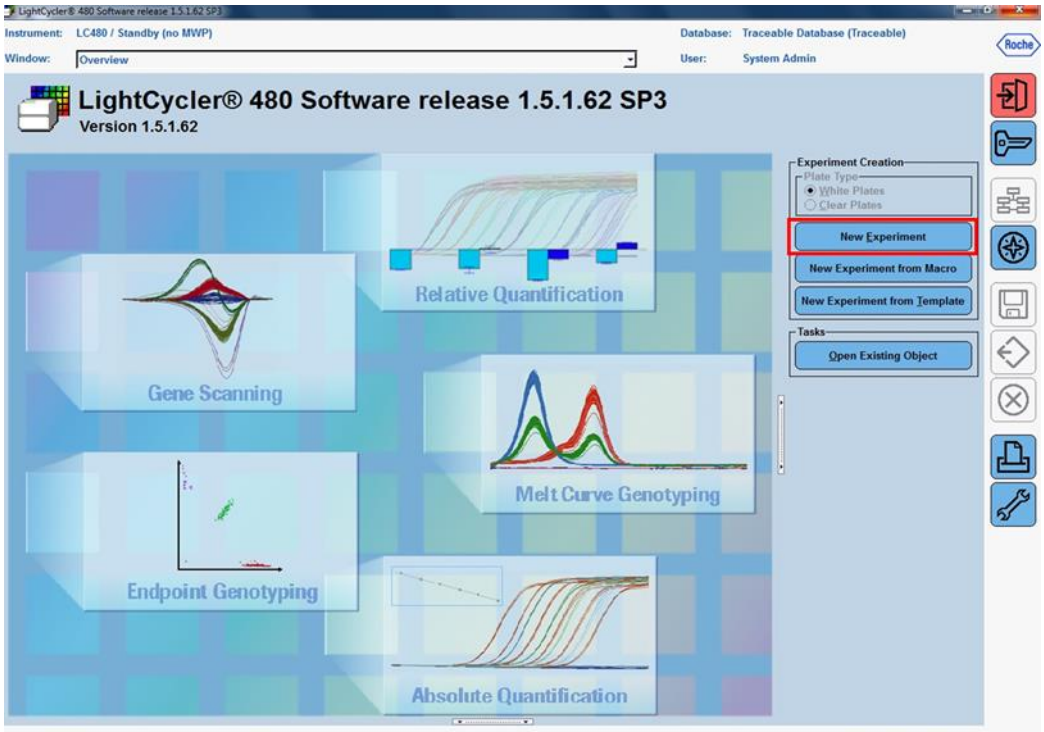
6. World Health Organization. Coronavirus disease (COVID-19) technical guidance: Laboratory testing for 2019-nCoV in humans. 3. Molecular assays to diagnose 2019-nCoV. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2

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

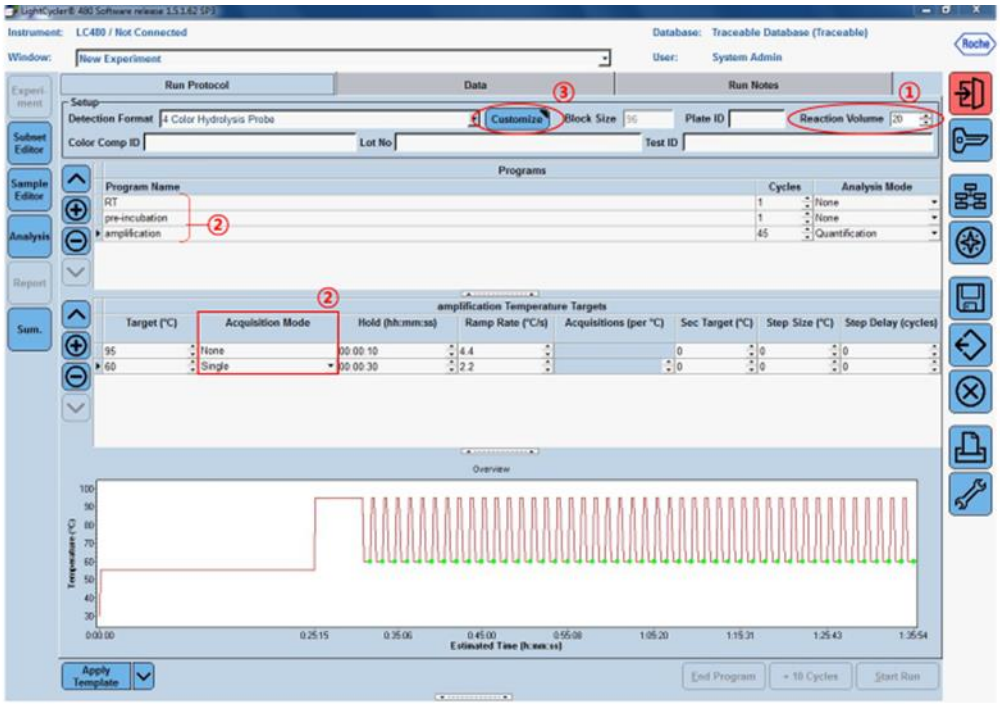
Appendix 1. Software Setting

① Light Cycler 480 (Roche, Product No. 05015278001)

i) Run a software and click “New Experiment”



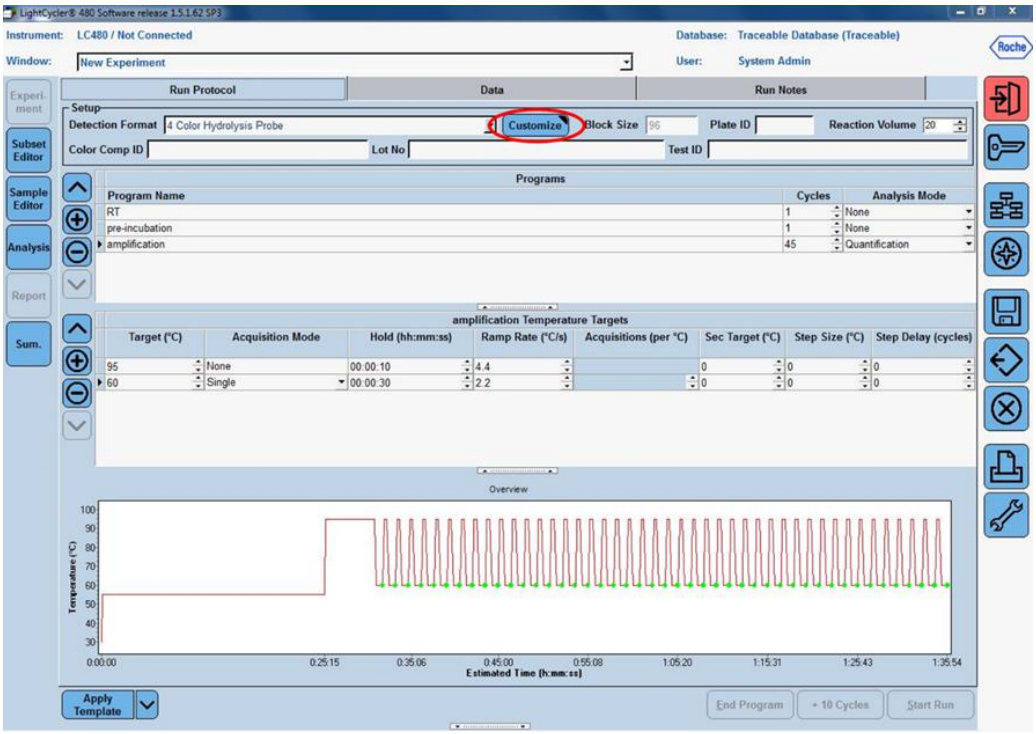
ii) Enter the reaction volume 20 ㎕ and modify PCR reaction conditions as below.

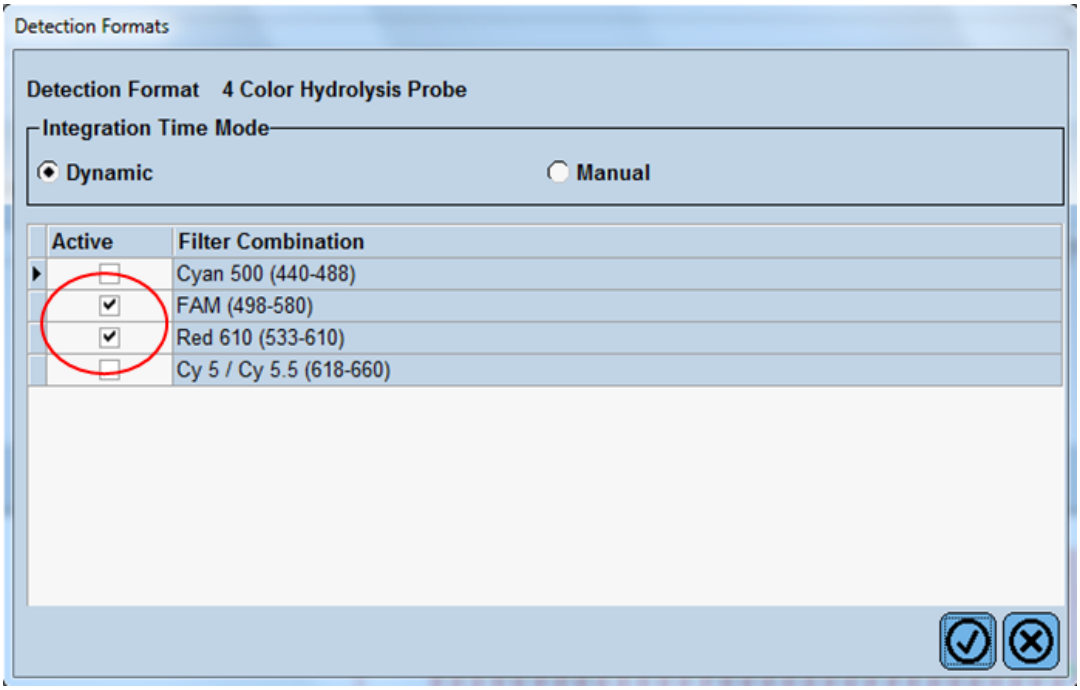


Step	Temperature	Time	Cycle
RT	55℃	25 min	1
Incubation	95℃	5 min	1
Amplification	95℃	10 sec	45
	60℃ *	30 sec	

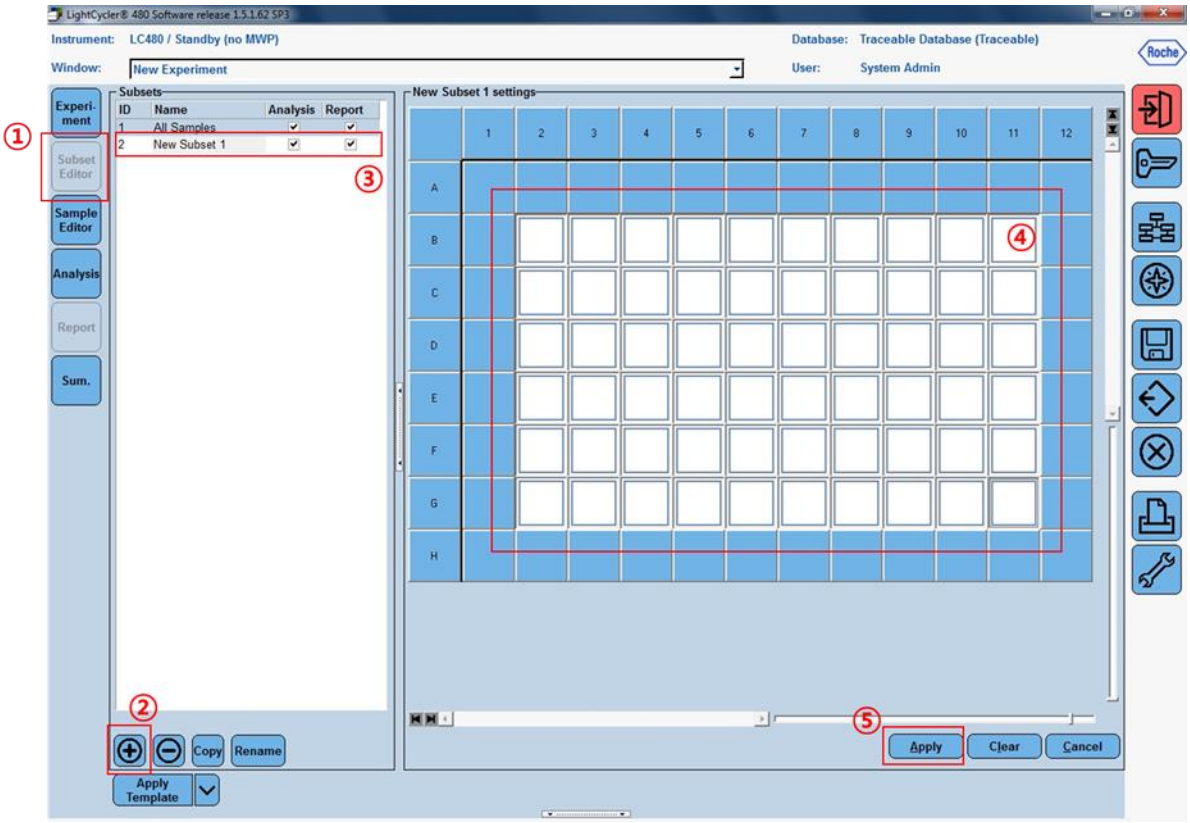
* Measure florescence at 60℃ (FAM and Red 610) channel

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

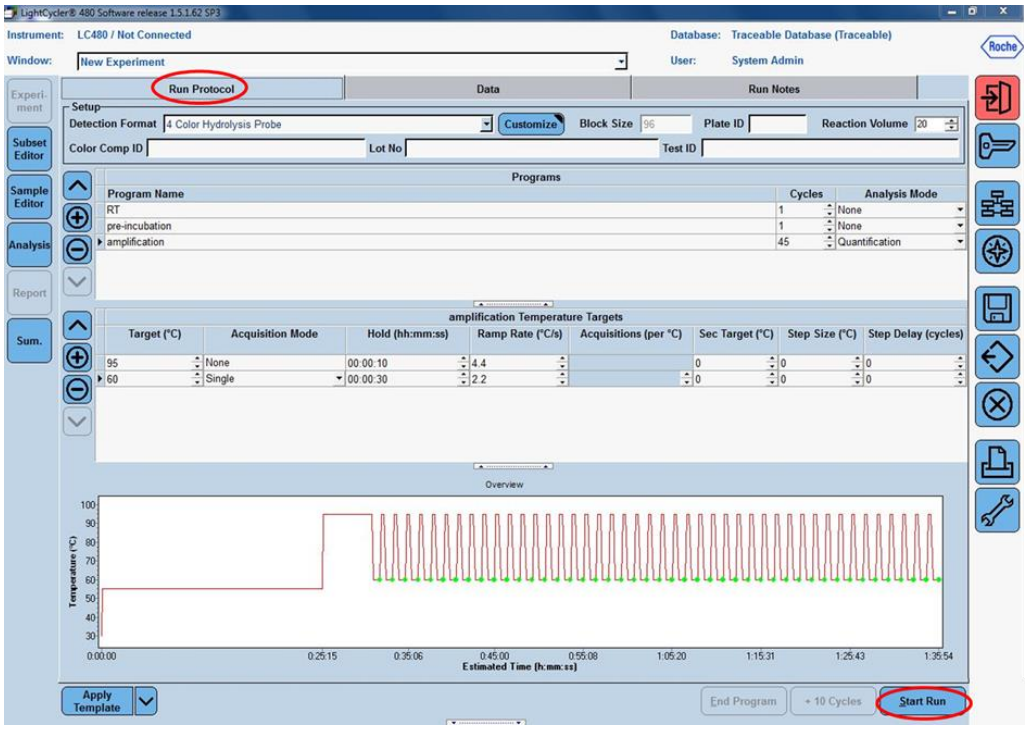




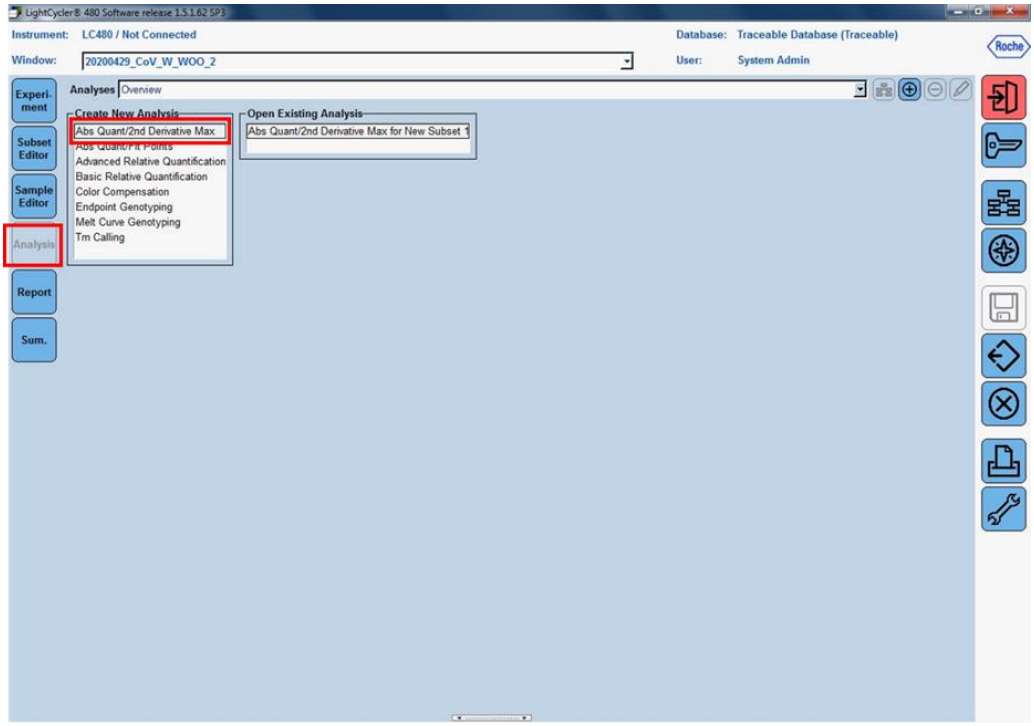
iv) Click “Subset Editor” and Define 96 well PCR plate layout on program.



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



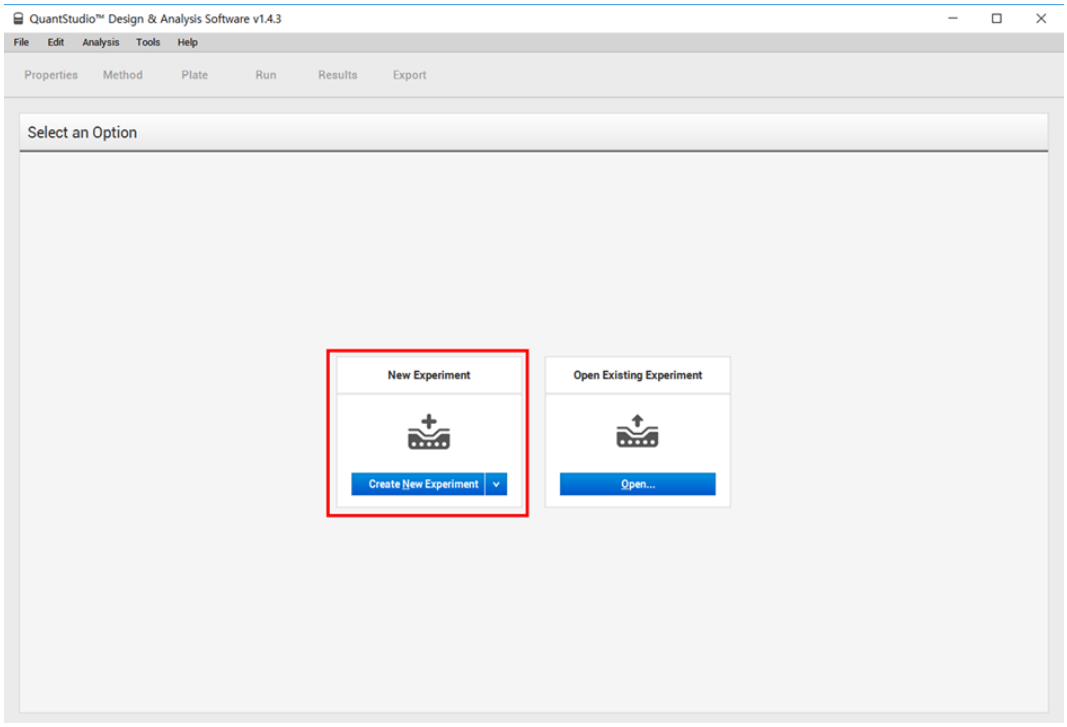
vi) For data Analysis follow the settings below table.



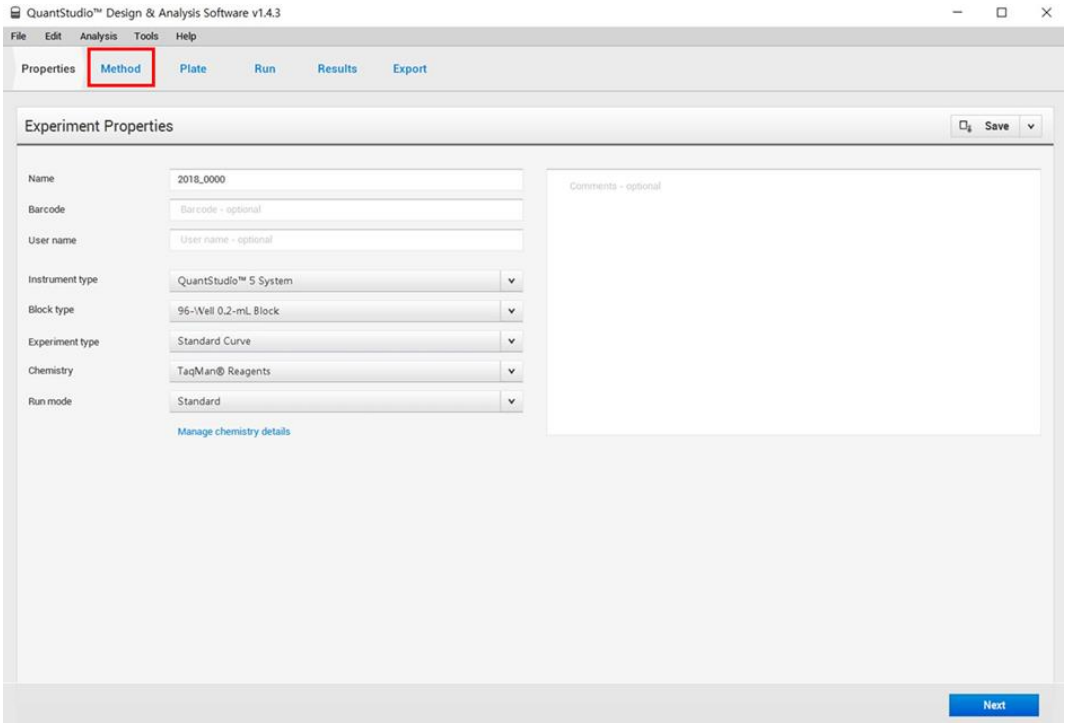
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene(FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene(FAM/Green)	300	50,000	15,000	0.1		3	15
IPC(Texas red/Red610/Orange)	100	20,000	5,000	0.1		3	15

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

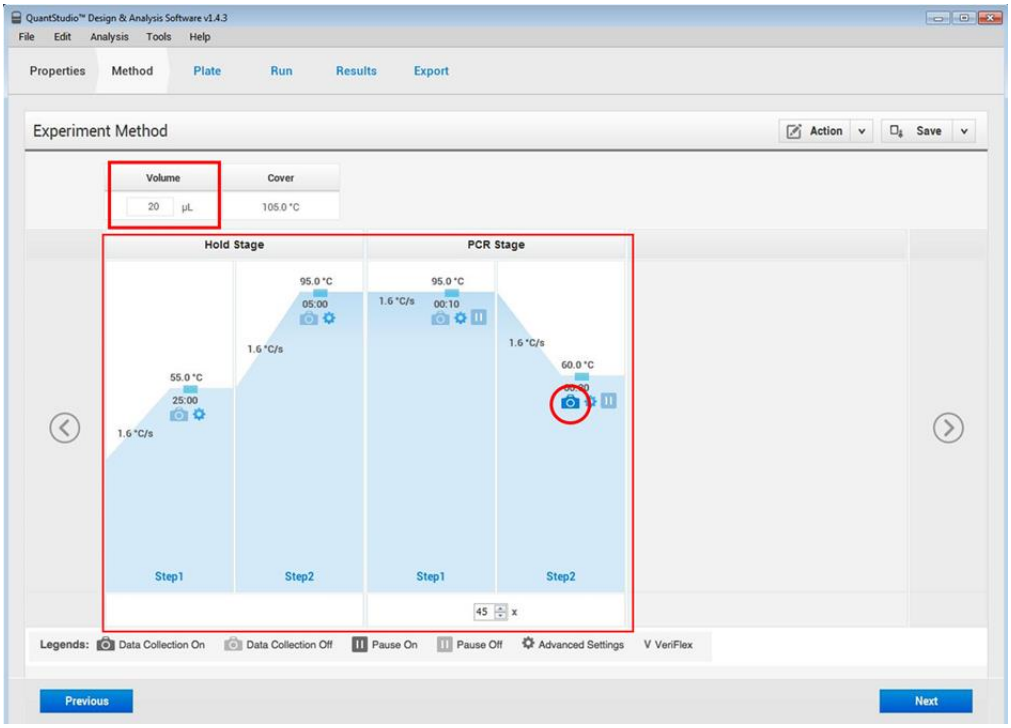
i) Run a software and click “Create New Experiment” of “New Experiment”



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



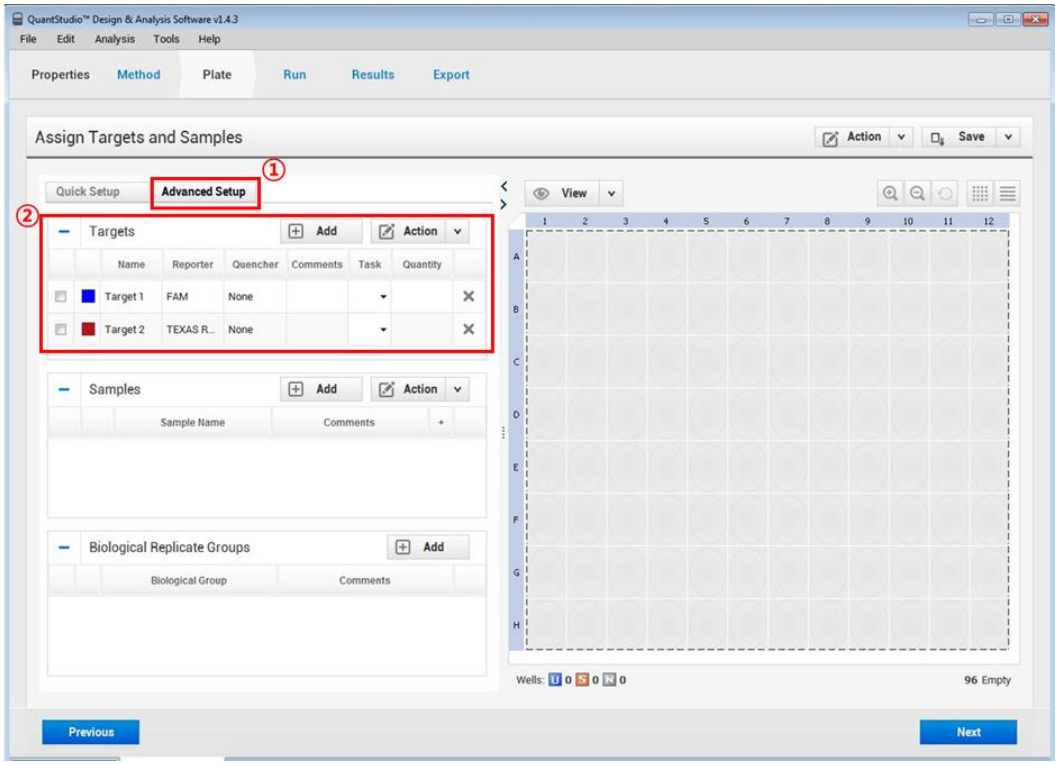
iii) Enter the reaction volume 20 ㎕ and modify PCR reaction conditions as below.



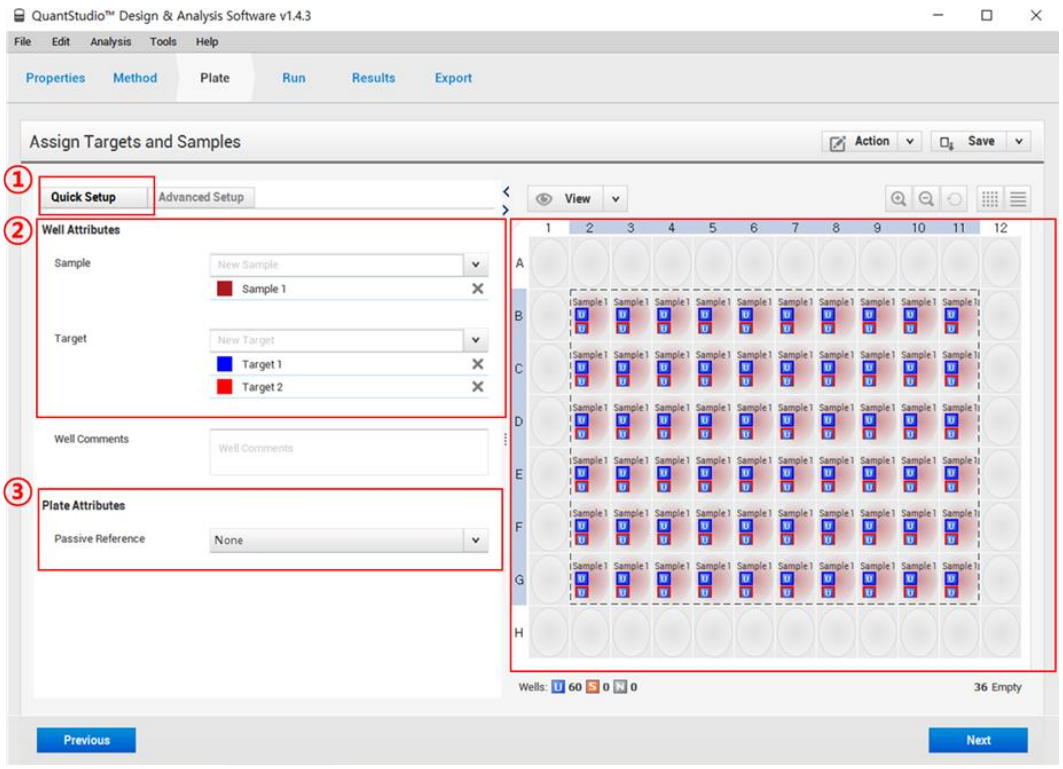
Step	Temperature	Time	Cycle
RT	55°C	25 min	1
Incubation	95°C	5 min	1
Amplification	95°C	10 sec	45
	60°C *	30 sec	

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

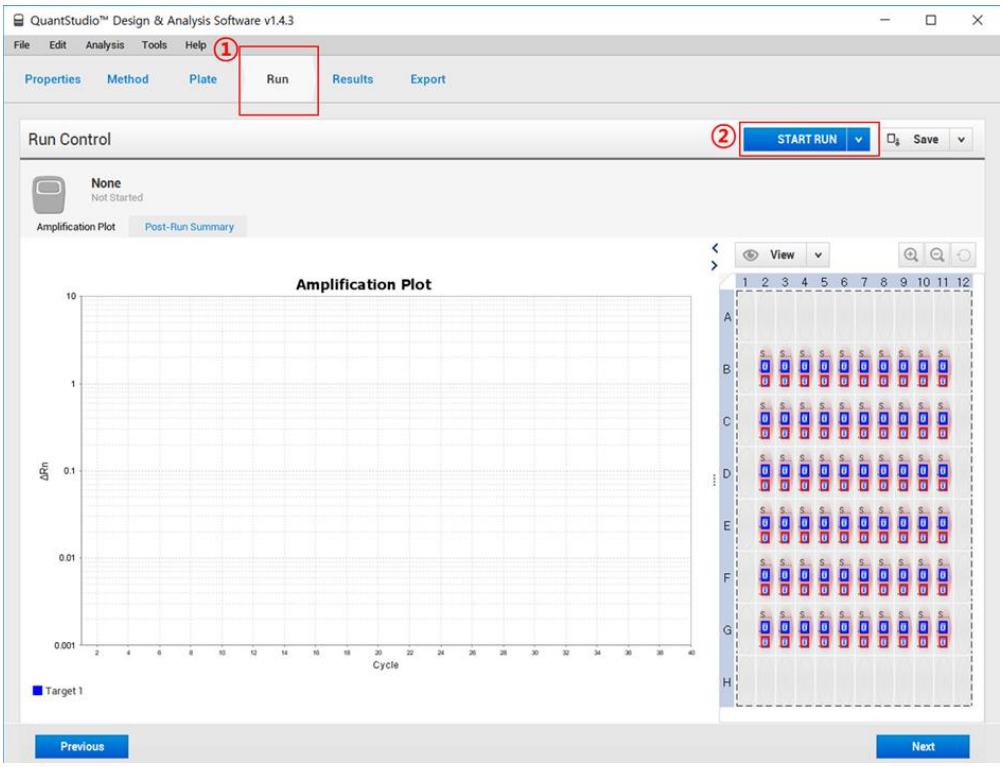
iv) Click “Plate” on the above menu bar and select “FAM” for Target1 and “TEX Red” for Target2 in “Advanced Setup”



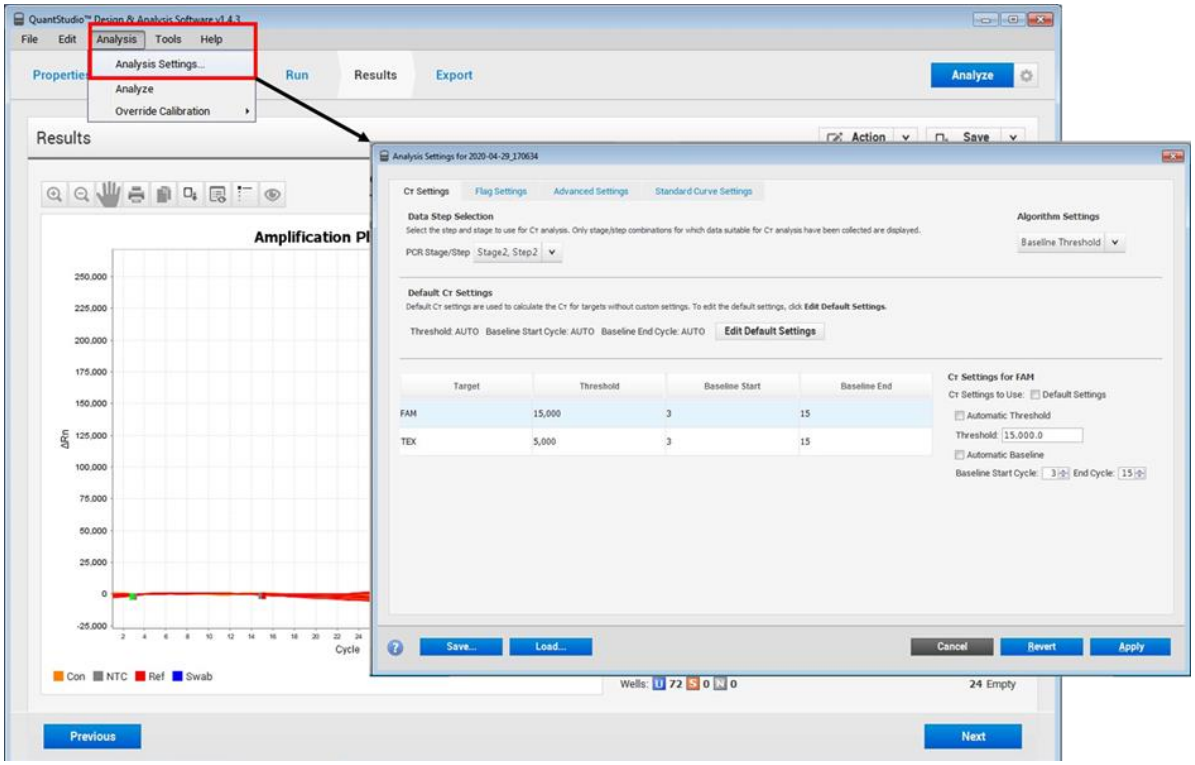
v) Click “Quick Setup” next to “Advanced Setup” and define 96 well PCR plate layout on program. Also, check the “Passive Reference : None”.



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



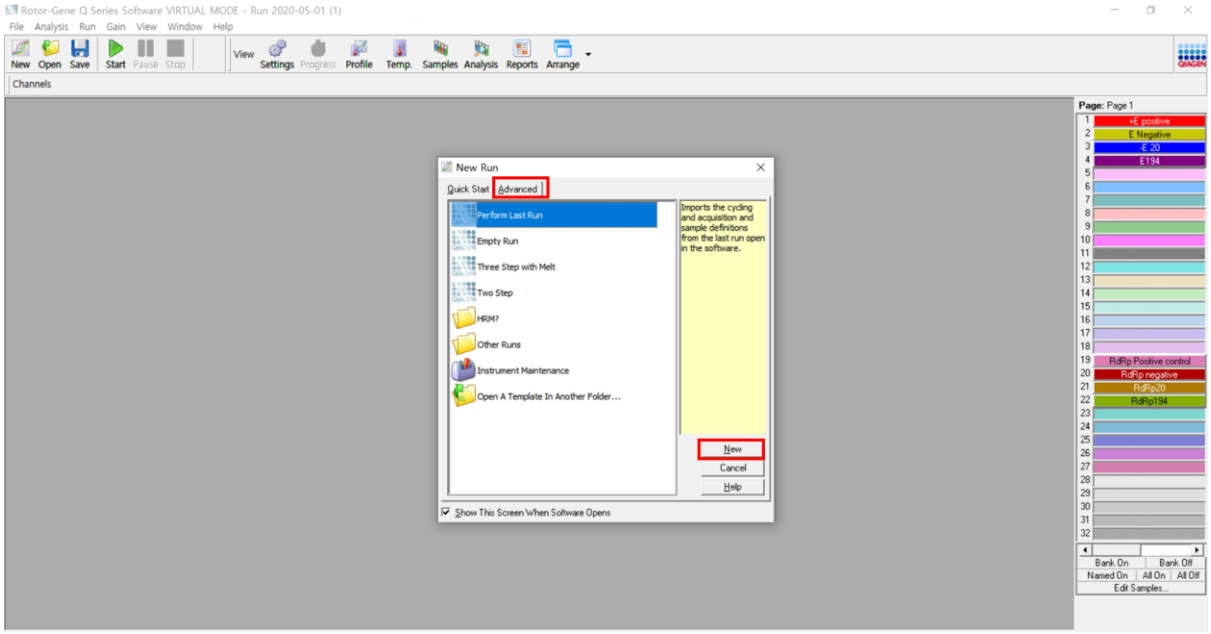
vii) For data Analysis follow the settings below table.



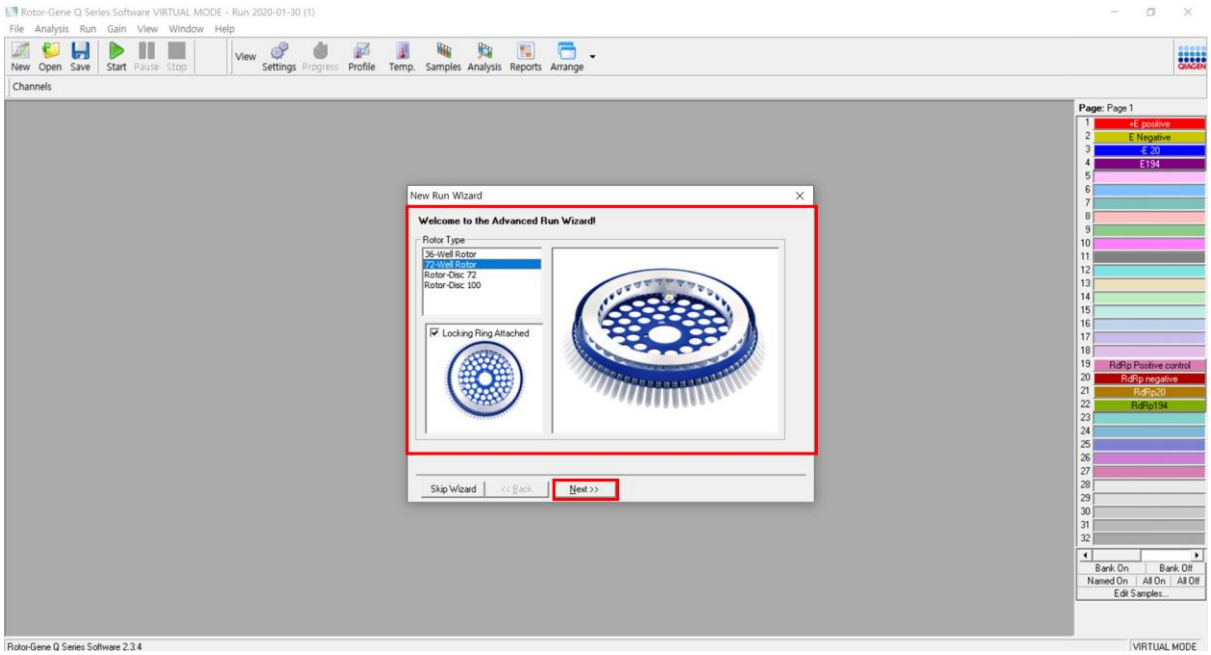
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene(FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene(FAM/Green)	300	50,000	15,000	0.1		3	15
IPC(Texas red/Red610/Orange)	100	20,000	5,000	0.1		3	15

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

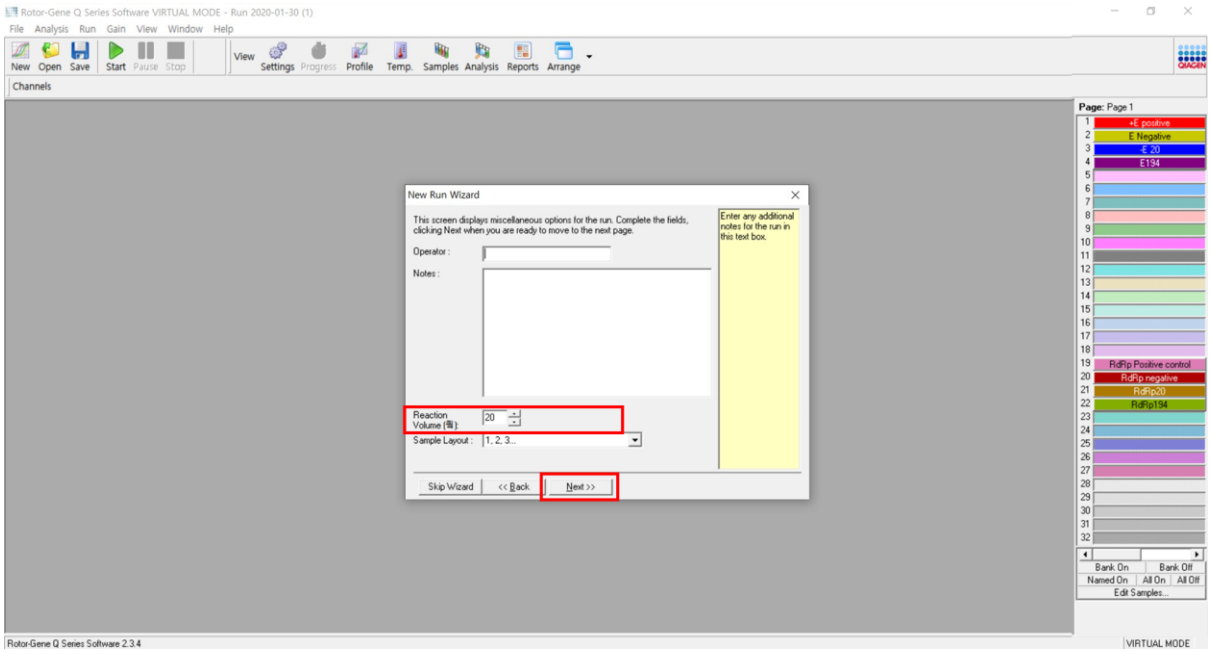
i) Run a software and click “Advanced” and click “New”.



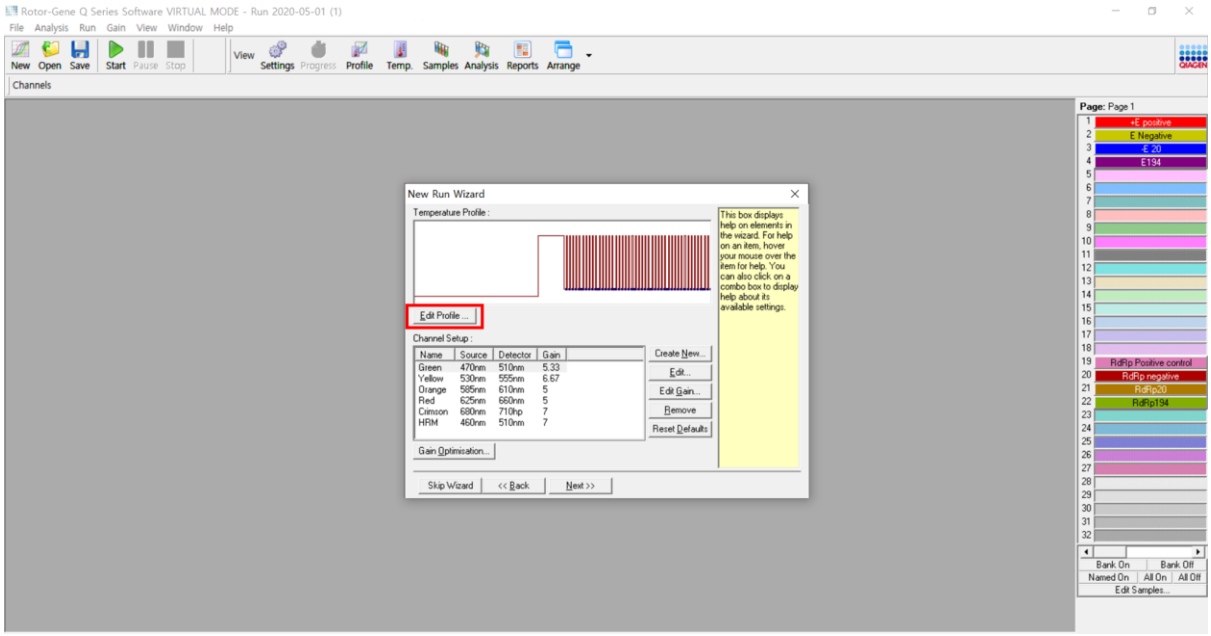
ii) Check the Rotor type and Click “Next”.



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



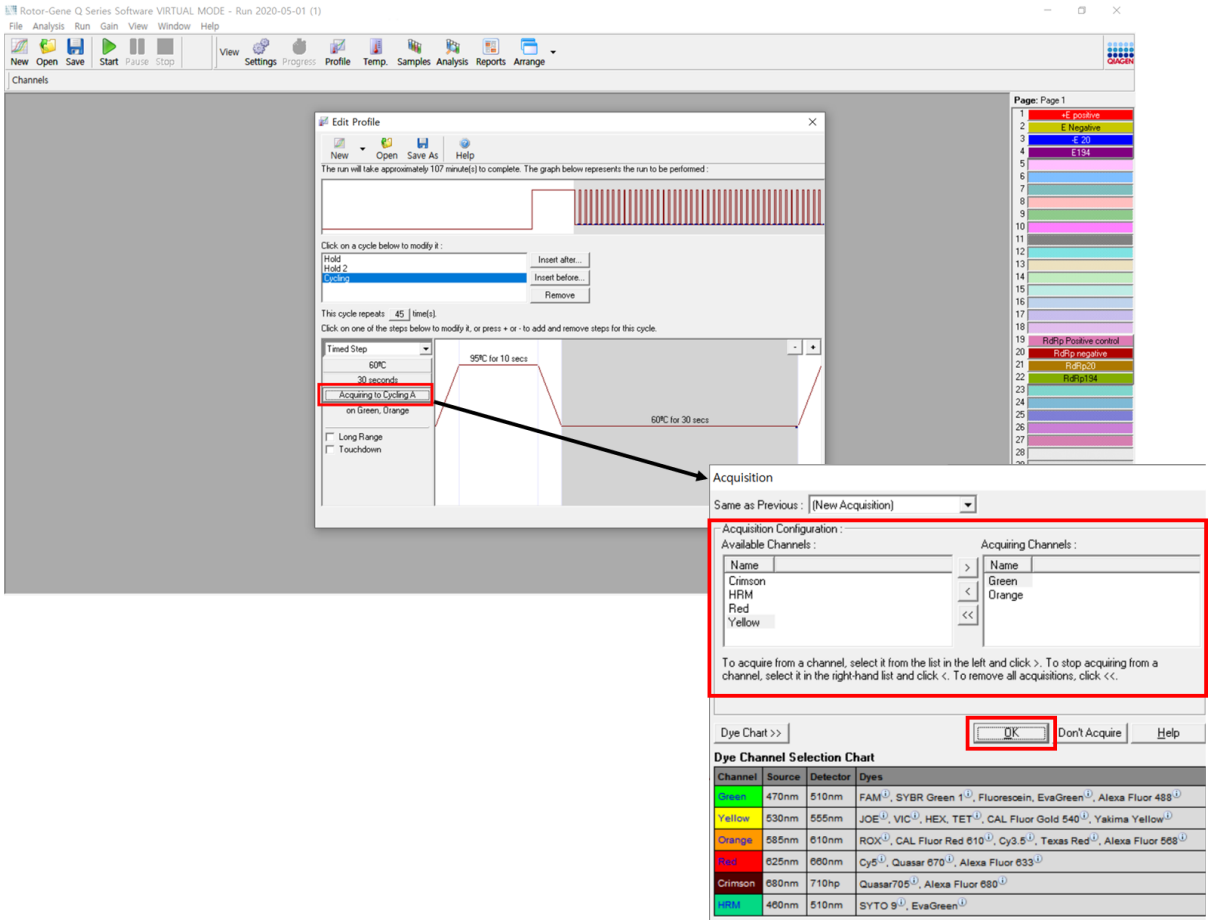
iv) Click “Edit profile” and modify PCR reaction conditions as below.



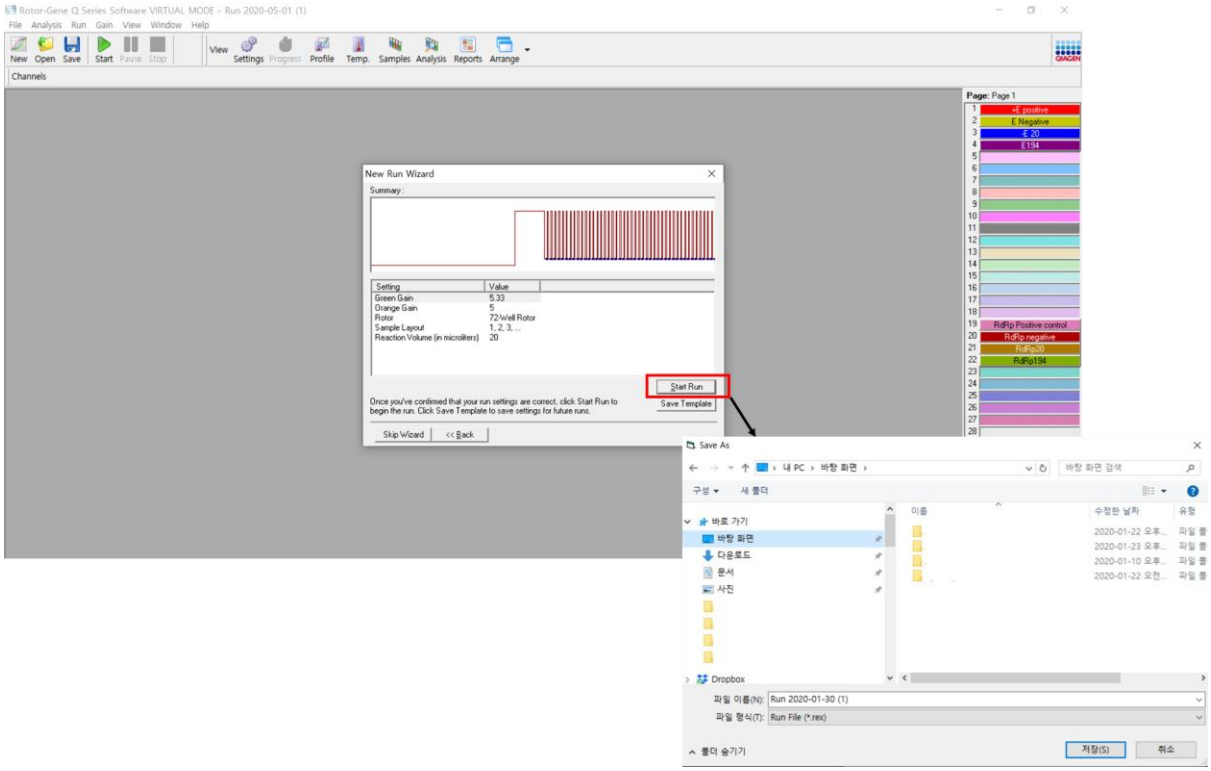
Step	Temperature	Time	Cycle
RT	55℃	25 min	1
Incubation	95℃	5 min	1
Amplification	95℃	10 sec	45
	60℃ *	30 sec	

* Measure florescence at 60℃ (Green and Orange) channel

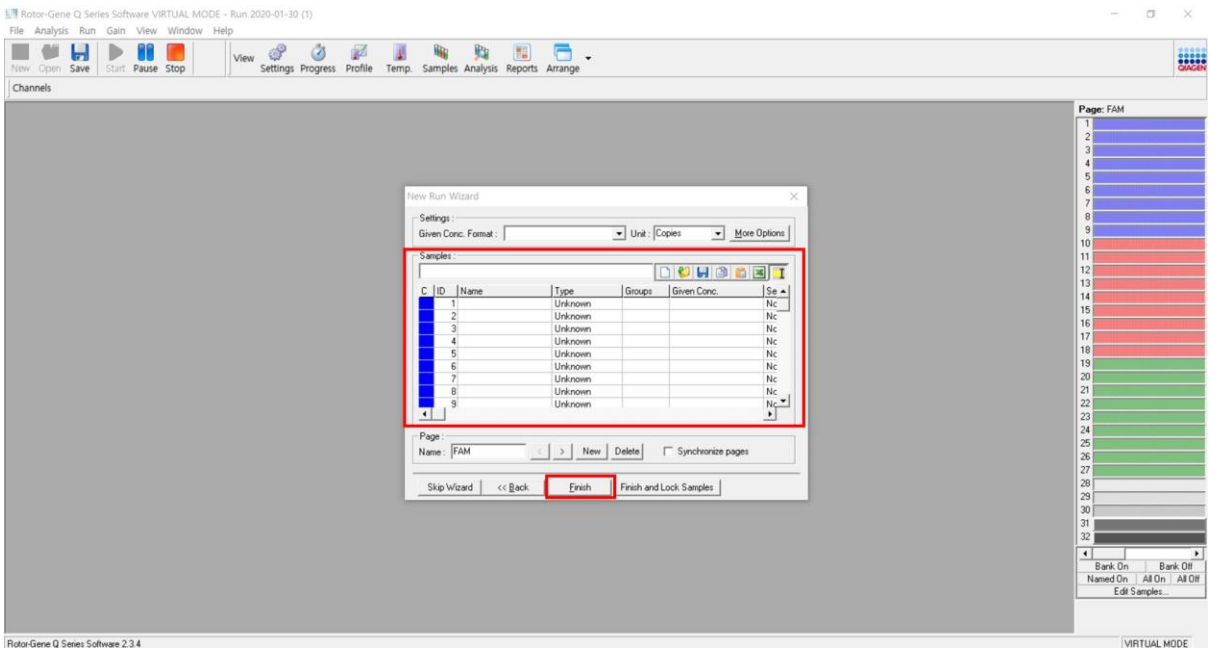
v) To measure fluorescence at 60℃, click the “Acquiring to Cycling A” and check the “Acquiring channels”, Green and Orange.



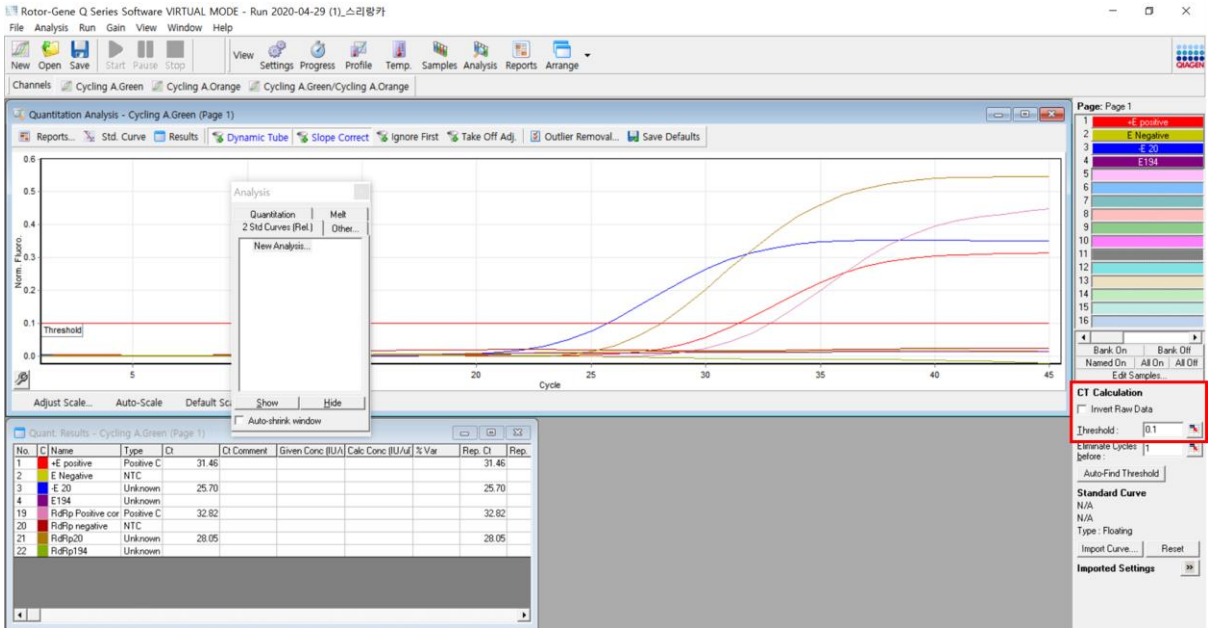
vi) Click “Start Run” and save the file.



vii) Define the samples and click “Finish”.



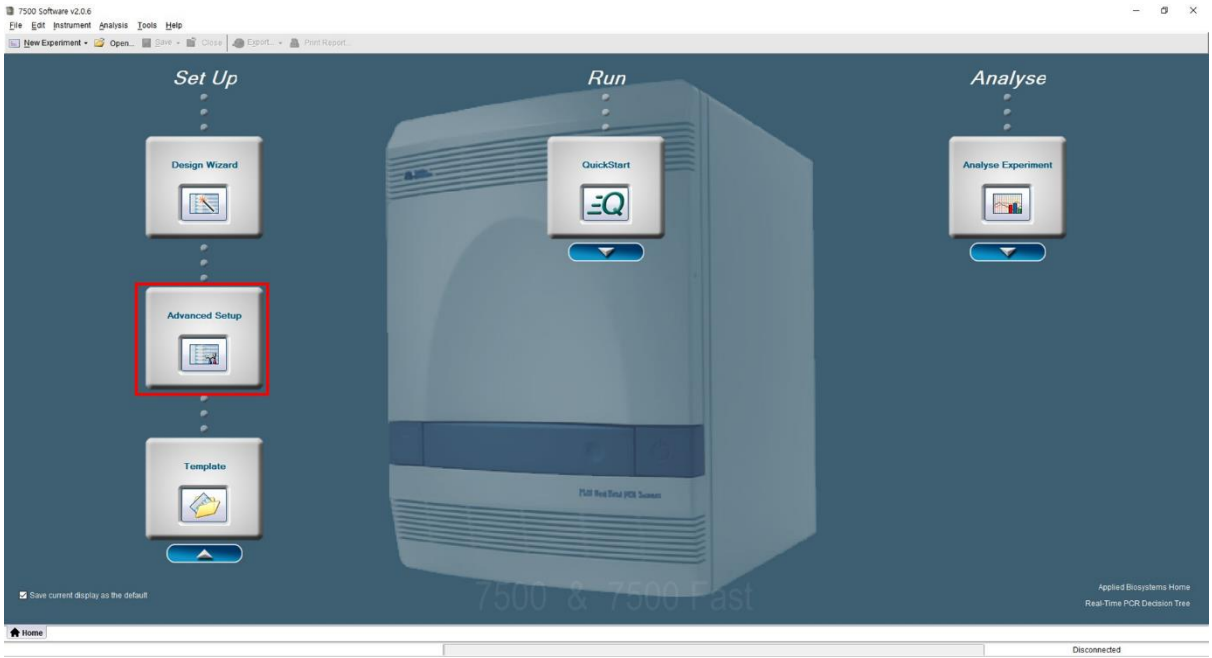
viii) For data Analysis follow the settings below table.



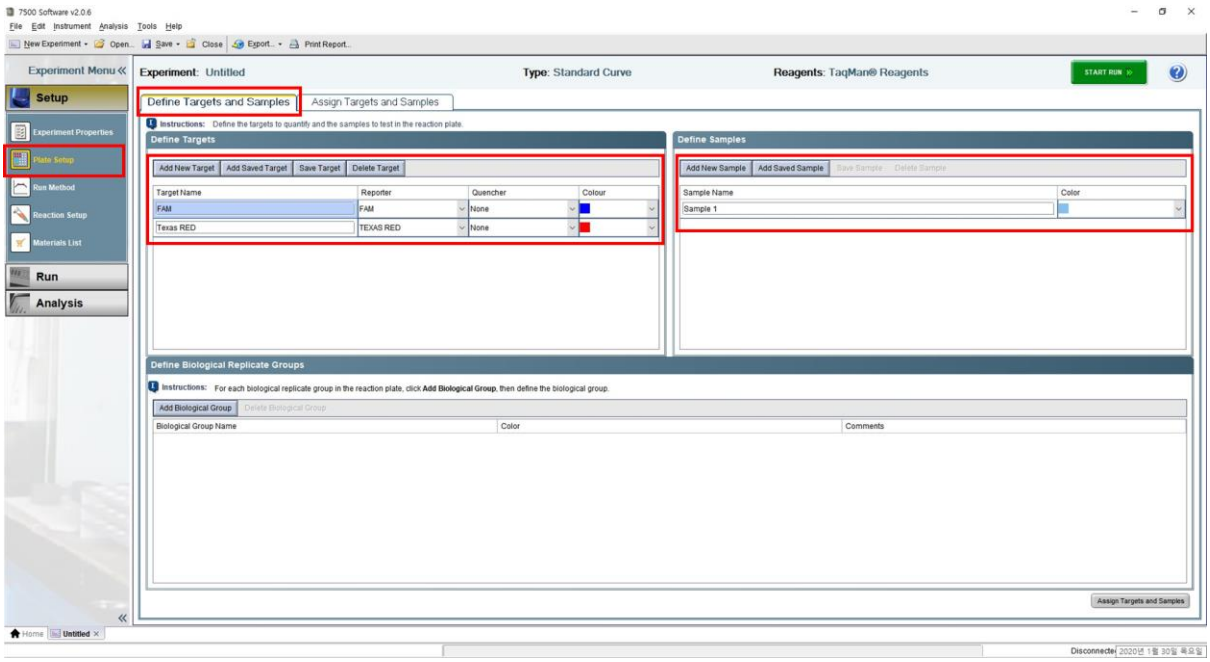
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene(FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene(FAM/Green)	300	50,000	15,000	0.1		3	15
IPC(Texas red/Red610/Orange)	100	20,000	5,000	0.1		3	15

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

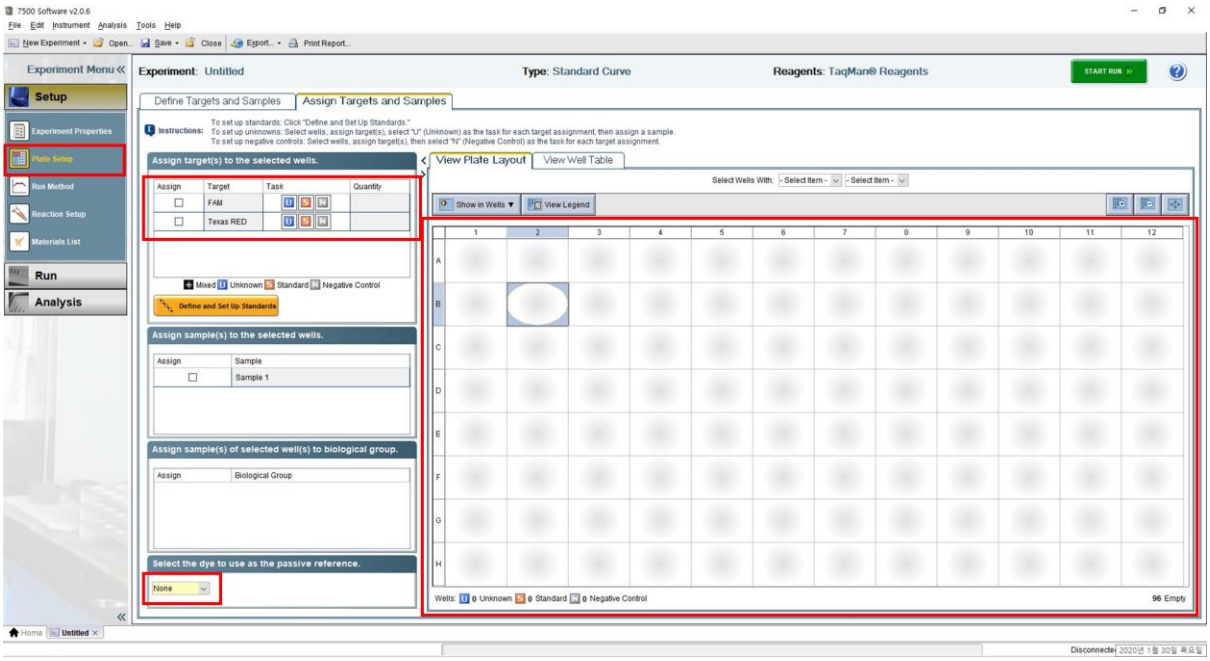
i) Run a software and click “Advanced setup”



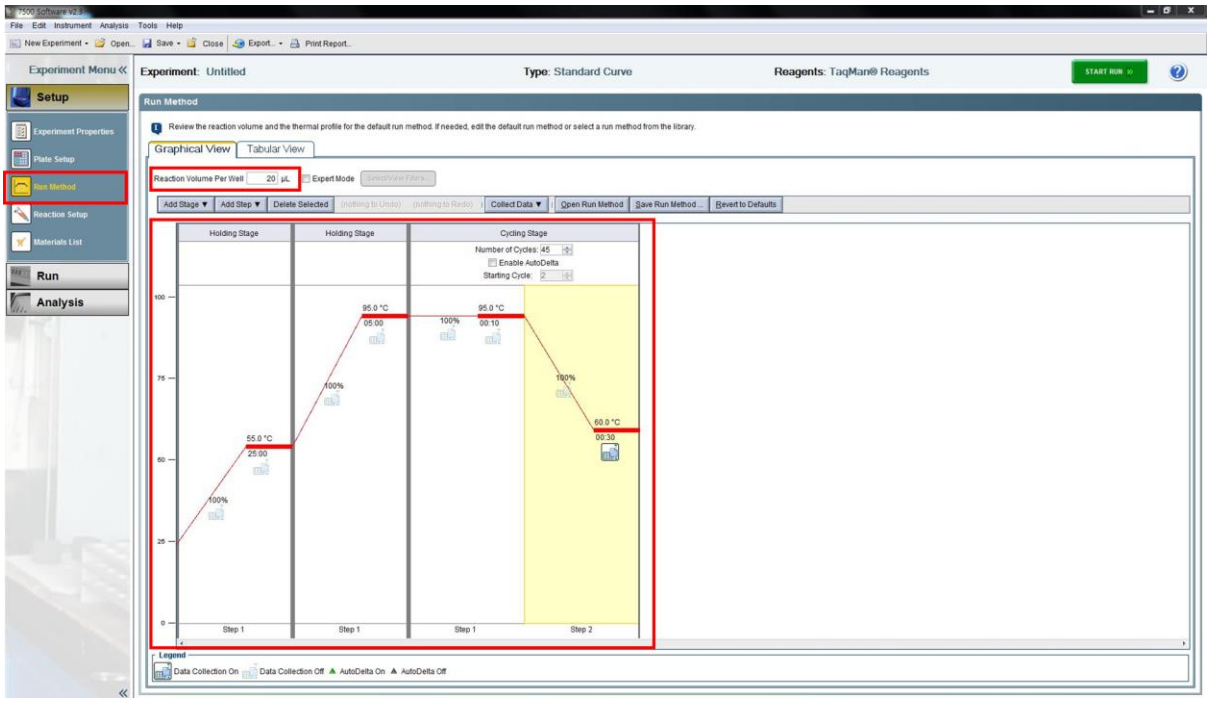
ii) Click “Plate setup” and select “FAM” for Target1 and “TEX Red” for Target2 in “Define Targets and Samples”



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



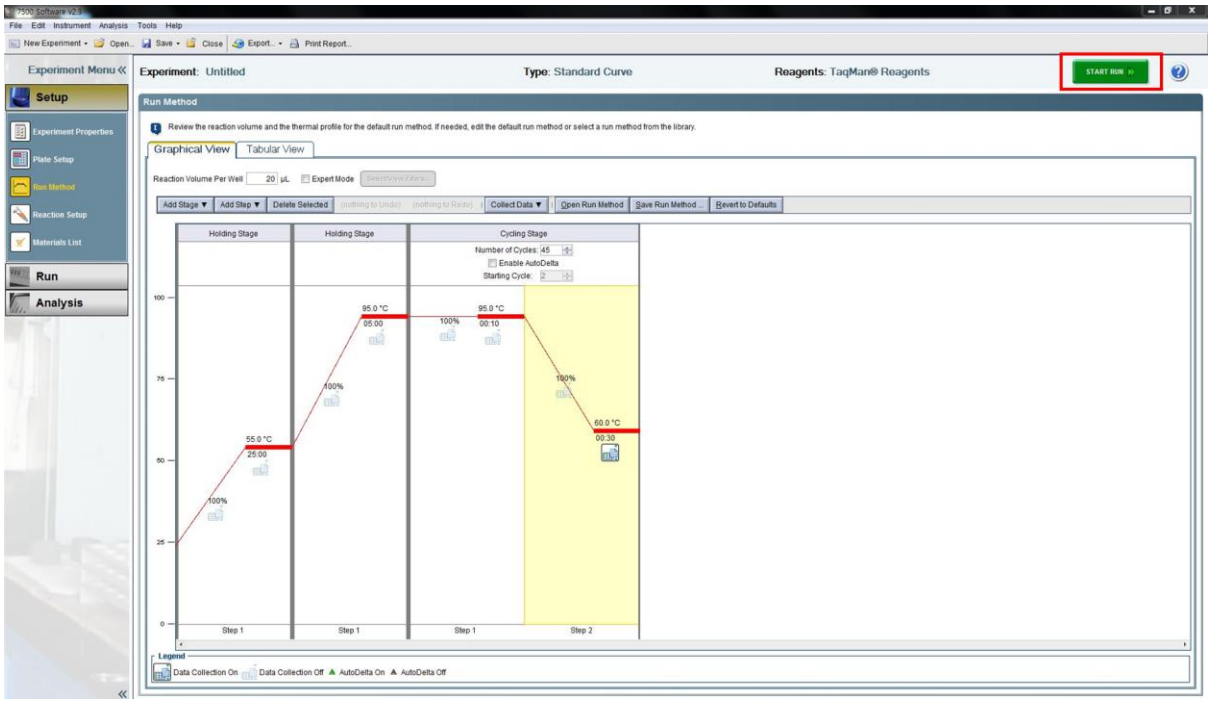
iv) Click “Run Method” and enter the reaction volume 20 ㎕ and modify PCR reaction conditions as below.



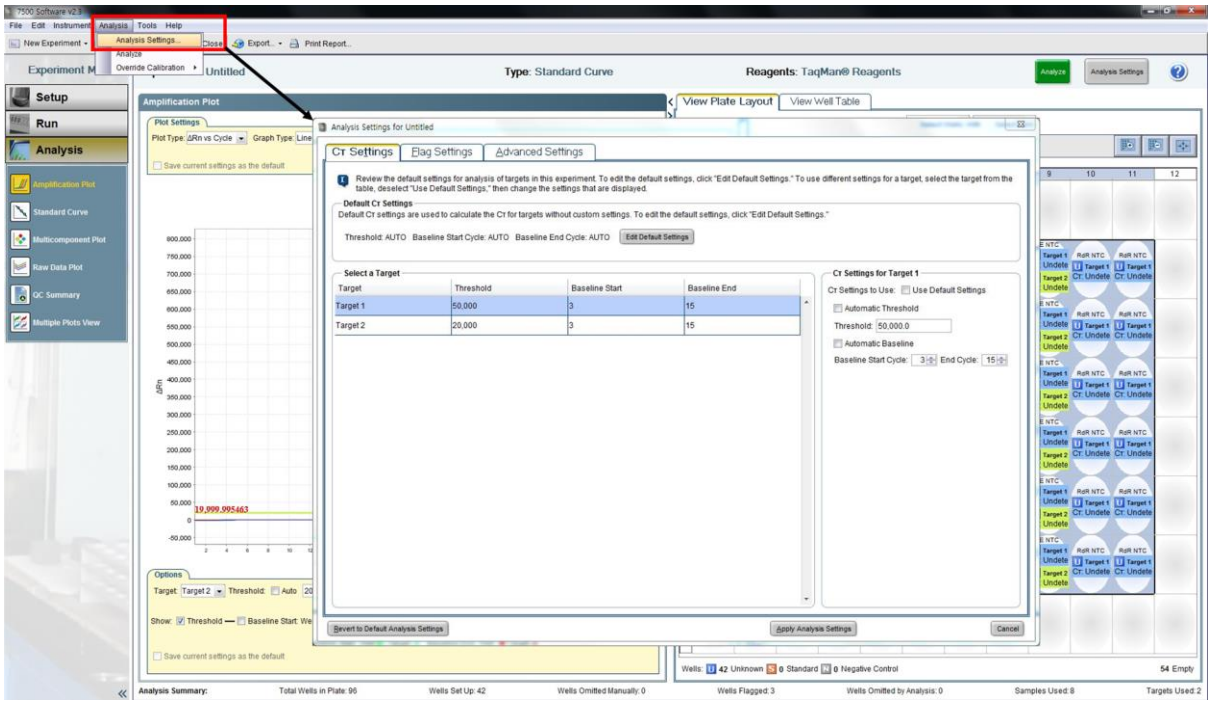
Step	Temperature	Time	Cycle
RT	55℃	25 min	1
Incubation	95℃	5 min	1
Amplification	95℃	10 sec	45
	60℃ *	30 sec	

* Measure florescence at 60℃ (FAM and TexRED) channel

v) Click “Start Run”.



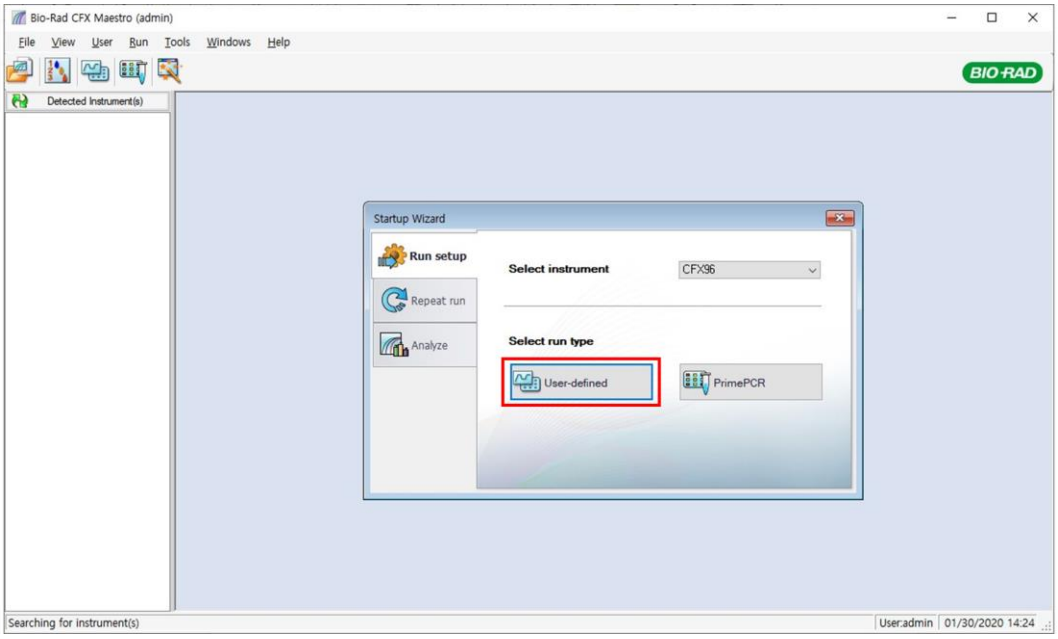
vi) For data Analysis follow the settings below table.



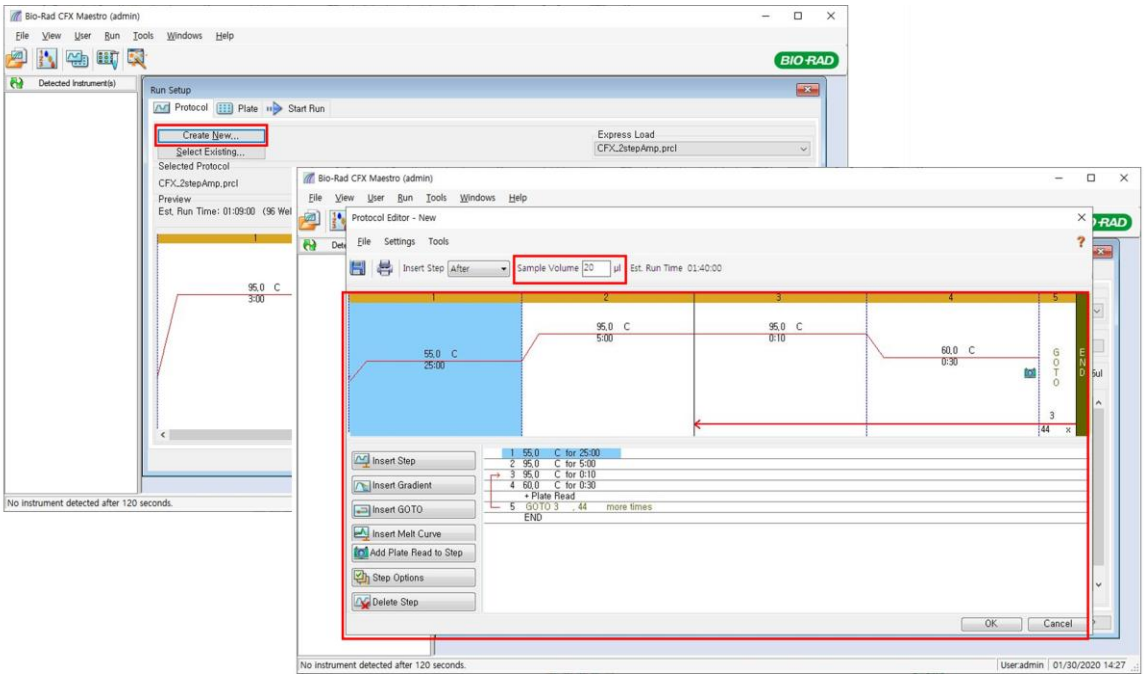
Target	Threshold					Baseline	
	CFX96	ABI 7500	ABI Quantstudio5	Rotor-Gene Q	LC480	Begin	End
E gene(FAM/Green)	300	50,000	15,000	0.1	Whether or not Ct is checked when setting Abs Quant/2nd Derivative Max	3	15
RdRp gene(FAM/Green)	300	50,000	15,000	0.1		3	15
IPC(Texas red/Red610/Orange)	100	20,000	5,000	0.1		3	15

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

i) Run a software and click “User-defined”



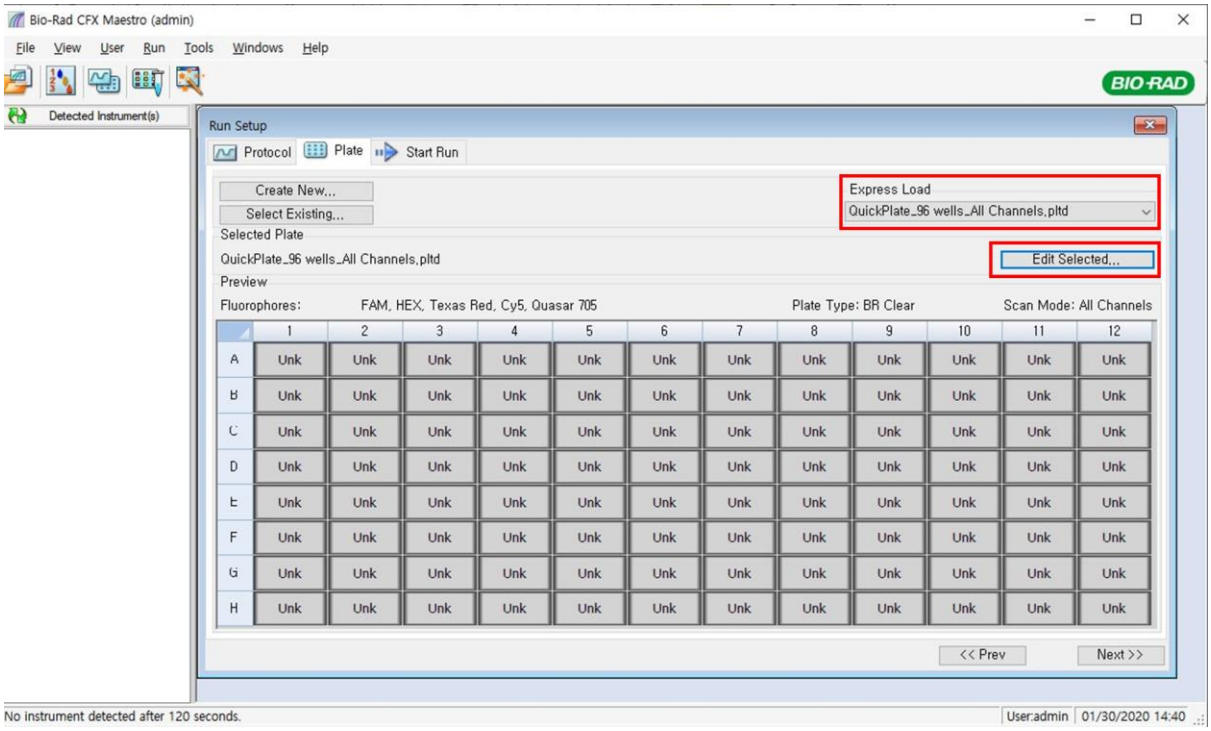
ii) Click “Create New” and enter the reaction volume 20 ㎕ and modify PCR reaction conditions as below.



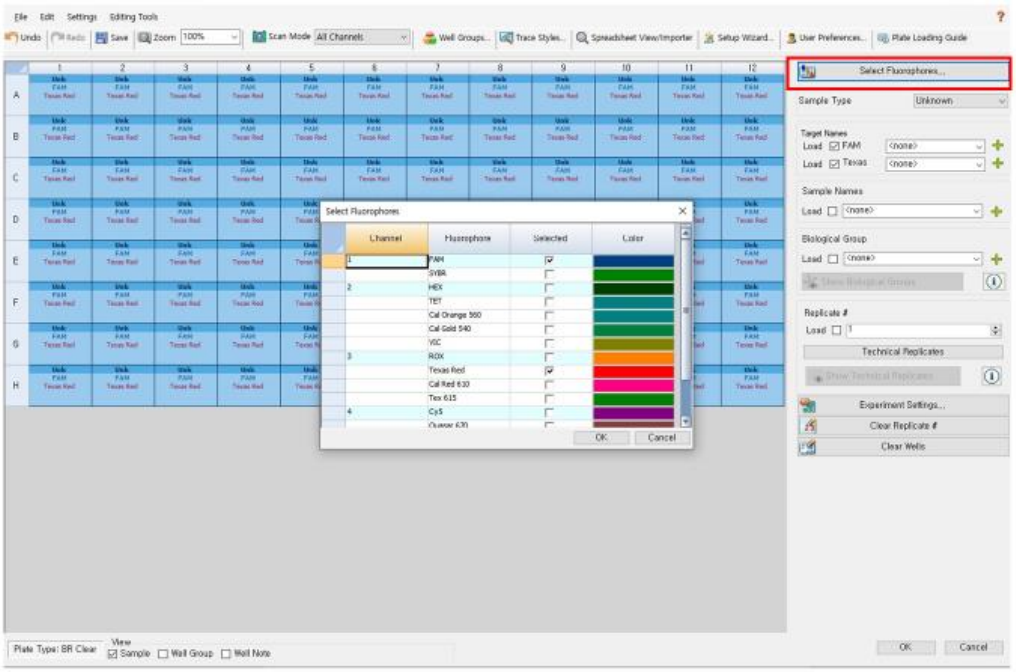
Step	Temperature	Time	Cycle
RT	55℃	25 min	1
Incubation	95℃	5 min	1
Amplification	95℃	10 sec	45
	60℃ *	30 sec	

* Measure florescence at 60℃ (FAM and TexRED) channel

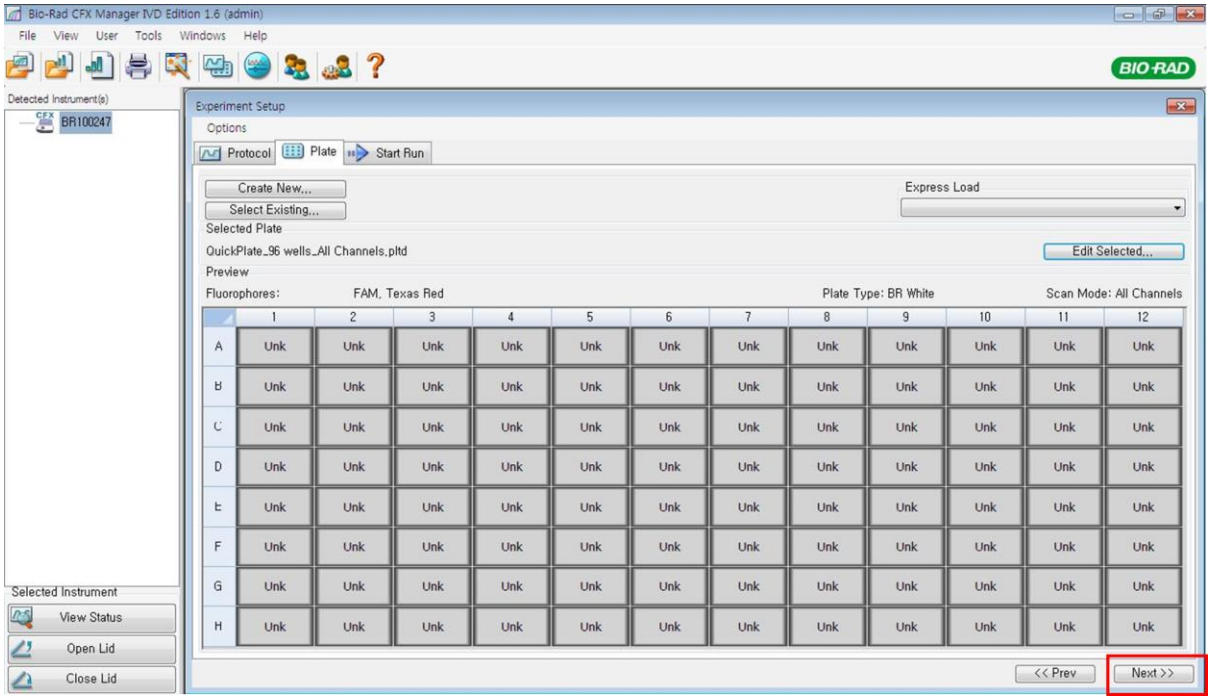
ii) Click “Plate” and check the “Express Load : QuickPlate_96 wells_All Channels.pltd” and click “Edit selected”.

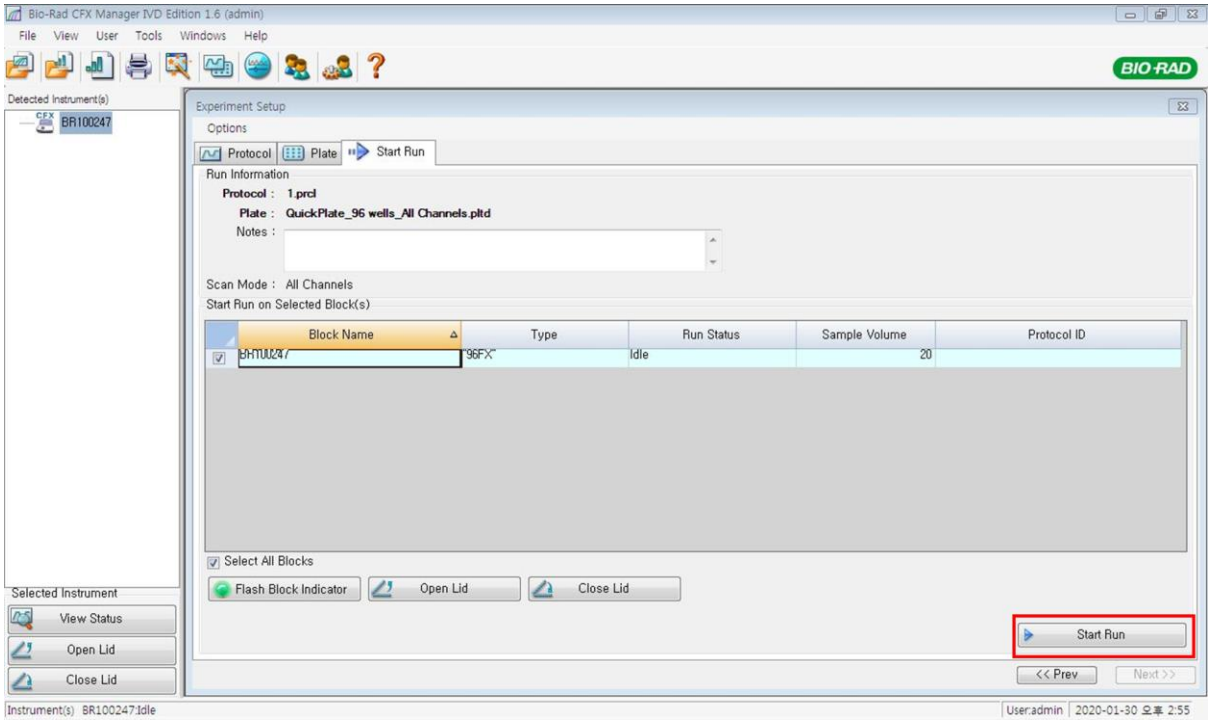


iii) Click “Select Fluorophores” and check FAM and Texas Red. Also, Define 96 well PCR plate layout on program.

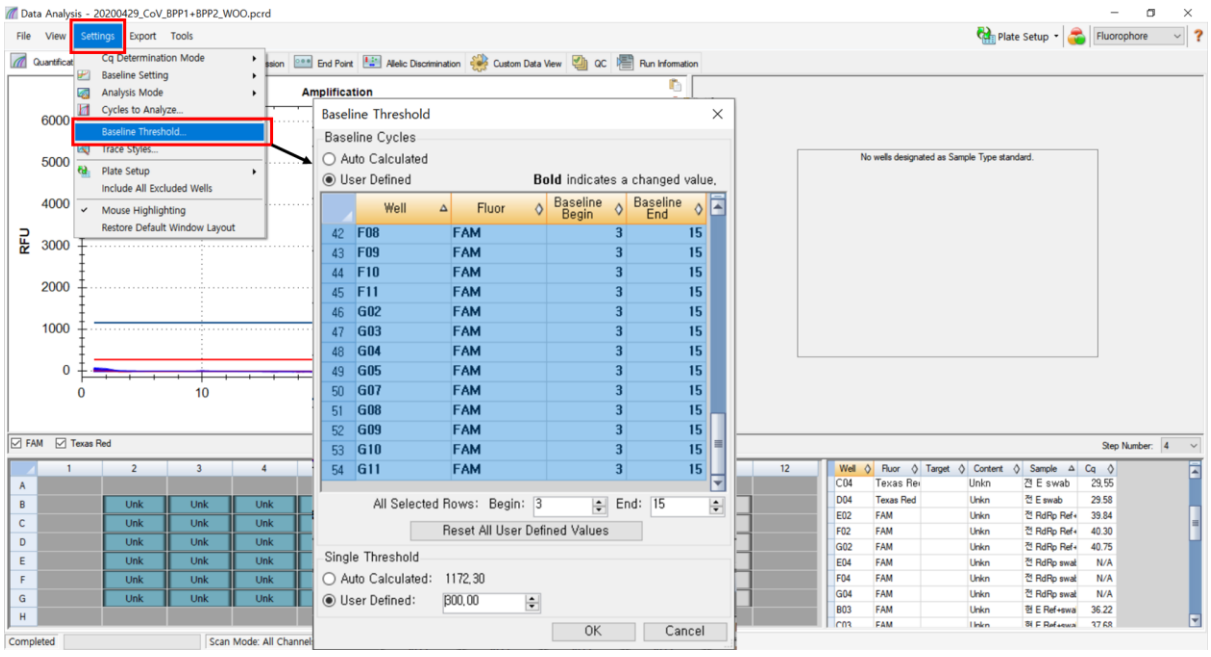


iv) Click “Next” and click “Start Run”





v) For data Analysis follow the settings below table.



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/Red610/Orange)	100	20,000	5,000	0.1		3	15