REF M24MD100T

1copy™ COVID-19 qPCR 4plex Kit

Σ 100

IVD CE

For *in vitro* diagnostic use
Prescription Use Only

Instructions for Use

Rev. Date : April 1, 2021

Doc.no. DR-M24-6033-E-02

1. Description

1copy™ COVID-19 qPCR 4plex Kit provides reagents for real-time RT-PCR that specifically target the E (Envelope) gene, the N (Nucleocapsid protein) gene and the RdRp (RNA dependent RNA polymerase) gene for the 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 4plex 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 providers.
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 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 the 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.

1copy™ COVID-19 qPCR 4plex 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

1copy™ COVID-19 qPCR 4plex 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 for E gene, Texas Red channel for N gene, VIC channel for RdRp gene, and Cy5 channel for the internal positive control (IPC). This kit uses dUTP and UNG enzymes to prevent contamination of amplified products.

4. Kit Contents (Materials Provided)

Kit contents	Cap color	Volume (100 Tests)
Master Mix	Red	1000 µℓ
Primer/Probe Mix (E gene, RdRp gene, N gene, IPC)	Brown (Amber tube)	100 µℓ
Control (E gene, RdRp gene, N gene, IPC)	Yellow	100 µℓ
DEPC DW	Clear	1000 µℓ

※ Control is positive control.
※ 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 specified in PCR instrument manufacturer's instructions
- 1.5 ㎖ micro tubes
- Sealing film
- Ice or cooling/cold block
- Microliter pipettes (1~10 µℓ, 10~100 µℓ, 100~1000 µℓ)
- Mini centrifuge (0.2 ㎖/0.5 ㎖ tubes, 10,000 rpm) or Benchtop centrifuge (1.5 ㎖ microcentrifuge and 96 well plate centrifuge) with rotor for 0.2 ㎖/0.5 ㎖ reaction tubes (capable of attaining 10,000 rpm)
- Vortex mixer
- 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)
- **qTOWER3 Real-Time PCR Thermal Cycler**
  (Analytik-jena, Product No. 844-00553-2, qPCR soft 4.0)

7. Warnings and Precautions

- 1copy™ COVID-19 qPCR 4plex Kit is for *in vitro* diagnostic use only.
- Do not eat, drink, smoke, or apply cosmetics and contact lenses where reagents and human specimens are handled.
- Follow safe laboratory procedures and handle all specimens as potentially infectious. 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 4plex Kit, as an *in vitro* diagnostics test, is only to be used on prescription. 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 for preparing test reagents, processing specimens and controls and conducting PCR are required in order to minimize contamination.
- All materials used in one area should remain in that area and should not be moved to 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.
- Use thawed contents after gently mixing and spinning down.
- Prepare mixtures of qPCR within a cooling/cold block or on ice.
- In case of contact with eyes, rinse immediately with water.
- Use a pipette to deposit samples directly into the reaction mixture 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.
- Do not introduce any foam or bubbles into the tubes when aliquoting Assay Mixtures. All PCR plates should be sealed prior to centrifugation and subsequent loading into the thermocycler to avoid any possible leakage and contamination.
- All lab workbenches 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. 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.

8. Reagent Storage and Handling

- Store the kit below -20°C.
- Expiration date for each 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/2019-nCoV/lab/lab-biosafety-guidelines.html

Follow specimen collection devices manufacturer instructions for proper collection 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. After obtaining specimen, place swabs immediately into sterile tubes containing 2-3 ㎖ of viral transport media or universal transport media.

The swab specimens 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.

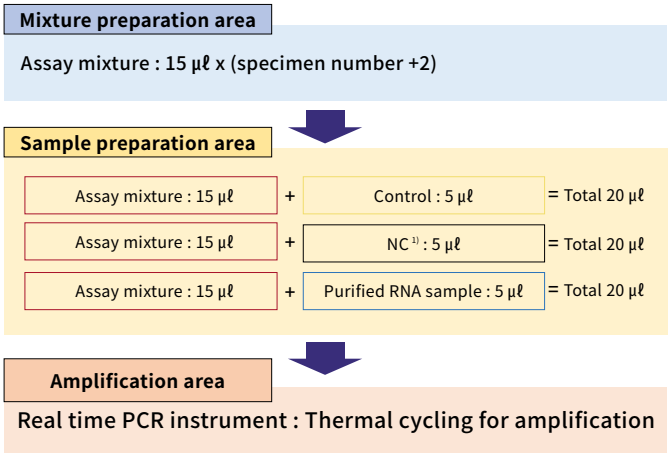
9.2 RNA extraction

* 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	40 µℓ

Schematic Workflow



1) NC is negative control(DEPC DW) supplied by manufacturer
Control should be run with each batch

9.3 RT-qPCR preparation

① Mixture Preparation

*Mixture should be prepared in area designated for mixture preparation to avoid contamination.

i) Prepare mixtures in PCR tubes according to the indicated volumes in the following table.

Mixture components	1 Reaction (Total volume : 15 µℓ)	Volumes for N specimens (µℓ)
Master mix	10 µℓ	10 x (N+2)
Primer Probe mix	1 µℓ	1 x (N+2)
DEPC DW	4 µℓ	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 should be prepared in area designated for sample preparation.
i) Add 5µℓ of the extracted RNA, control, 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 4plex Kit.

① Enter the reaction volume as 20 µℓ and modify PCR conditions as below.

Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

* Measure fluorescence at 60°C
* For ABI 7500, set the amplification time for 60°C as 28 sec.
* Time taken to run each PCR cycle may vary depending on the instrument used

② Select the type of measurement fluorescence.

Target	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene Q	LC480
E gene	FAM			FAM	Green	FAM
RdRp gene	VIC			JOE	Yellow	VIC
N gene	Texas Red			Texas Red	Orange	Red610
IPC	Cy5			Cy5	Red	Cy5

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

FAM, Texas Red, VIC and Cy5 are the most commonly used fluorescent dyes, and please note that their names may vary for different PCR machines. Confirm that the PCR machines have corresponding filters prior to testing.

10. Quality Control

* Control and negative control(NC) should be run with each batch.

- DEPC DW provided in this kit is used as a negative control to evaluate if any contamination of the reaction mixture is present. 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 contains E gene, N gene, RdRp gene and IPC plasmids and it is used as a positive control. Positive controls are essential in assessing the amplification detection process, primer and probe integrity and run validity. Each positive control should produce a positive result for the applicable target (Ct value ≤40). If expected positive reactivity is not achieved, the run should be invalidated and repeated with a new aliquot of control.
- IPC should be present in each clinical specimen, and is co-purified with target SARS-CoV-2 virus. Therefore, the IPC can be used as a sampling and extraction control.
  IPC needs to determine the validity of both sampling and extraction processes. The IPC must be detected (Ct value ≤40) 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 Control, IPC and clinical specimens, the cut off value for each applicable target to be considered "detected" (+) is a Ct value ≤40.

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

Ct values above 40 for FAM, Texas Red, VIC and Cy5 may be a result of unspecific amplification.
The analytical cut-off value for this product is 40, but this value can be readjusted, depending on the environment of the laboratory.

※ Set threshold values and baseline

All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

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

11.2 Controls interpretation

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

Control				Negative Control				Interpretation
FAM	VIC	Texas Red	Cy5	FAM	VIC	Texas Red	Cy5	
+				-				Pass
+/-			-	-				Control Failure / System stability 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 (-)

11.3 Patient specimen interpretation

E gene assay (FAM)	RdRp gene assay (VIC)	N gene assay (Texas Red)	IPC (Cy5)	Interpretation
+			+/-	Positive for SARS-CoV-2[a)]
+/-	+	-	+/-	Presumptive Positive for SARS-CoV-2[b)]
+	-	+/-		
-	+/-	+		
-			+	Negative for SARS-CoV-2
-			-	Invalid Result[c)] Repeat extraction and RT-PCR, if result obtained from a repeated test is invalid, collection of new specimen is recommended.

a) If sufficient biological samples (clinical matrix) are not collected and viral load is high, E gene, N gene, and RdRp gene can be positively detected even if IPC is confirmed as negative.
b) A positive result in single, or 2 target results may be suggestive of
1) a sample at concentrations near or below the limit of detection of the test,
2) a mutation in the 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) carry-over contamination by control or patient samples, or
5) other factors.
c) Invalid result due to potential sampling error or inhibition.
※ 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 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 viral RNA during shipping/storage
- Using of unauthorized extraction or assay reagents
- 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 effects of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.
· Negative results do not preclude SARS-CoV-2 infection and should not be the sole basis of a patient management decision.
· Positive result indicates the detection of nucleic acid from SARS-CoV-2, but do not reflect the viral load in tested specimens.
· Nucleic acids 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 1copy™ COVID-19 qPCR 4plex 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 1copy™ COVID-19 qPCR 4plex Kit may give in inaccurate results.
· As with any molecular test, mutations within the target regions of 1copy™ COVID-19 qPCR 4plex 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 1copy™ COVID-19 qPCR 4plex 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 Limit of Detection (LoD)

Studies were performed to determine the analytical limit of detection (LoD) of the 1copy™ COVID-19 qPCR 4plex Kit. The LoD of 1copy™ COVID-19 qPCR 4plex Kit was established using one lot of reagents.

The RNA reference material for this experiment was AccuPlex™ SARS-CoV-2 Verification Panel (Seracare, Cat. No. 0505-0168, stock concentration 10^3, 10^4, 10^5 copies/mℓ as determined by digital PCR). The reference material was serially diluted into pooled nasopharyngeal/oropharyngeal swab matrix.

The LoD is defined as the lowest concentration at which 23/24 replicates are positive for each assay target.

The LoD for 1copy™ COVID-19 qPCR 4plex Kit is shown in the following table for each assay target and PCR instruments.

Target	CFX96	7500	Quantstudio5	LC480	Rotor Gene-Q	qTOWER3
N gene (copies/mℓ)	400	400	400	400	400	400
E gene (copies/mℓ)	400	400	400	400	400	400
RdRp gene (copies/mℓ)	400	400	400	400	400	400

13.2 Inclusivity

The inclusivity of the 1copy™ COVID-19 qPCR 4plex Kit was evaluated using in silico analysis of the assay primers and probes in relation to 3,728 SARS-CoV-2 sequences available in the GISAID gene database for three targets, E gene, N gene and RdRp gene.

For the E gene, 1copy™ COVID-19 qPCR 4plex Kit have 18 sequence single-point mutations in a total of 19 genomes, four-point mutations in 1 genome. For the N gene, 1copy™ COVID-19 qPCR 4plex Kit have 19 sequence single-point mutations in a total of 21 genomes, and two-point mutations in 1 genome and three-point mutations in 1 genome. For the RdRp gene, 1copy™ COVID-19 qPCR 4plex Kit have 8 sequence single-point mutations. None of these mismatches found for three targets are predicted to have a negative impact on the performance of the assay, given the location of the mutations in the primer and probe regions respectively. These mutations are not predicted to adversely affect the probe and primer binding to the sequences or reduce assay efficiency.

13.3 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 E gene, N gene and RdRp gene primers and probes in 1copy™ COVID-19 qPCR 4plex 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. *In silico* analysis suggests cross-reactivity of the 1copy™ COVID-19 qPCR 4plex Kit primers/probe sets for the E gene targets with only SARS-coronavirus.

To further evaluate the potential for cross-reactivity of 1copy™ COVID-19 qPCR 4plex 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, N gene and RdRp gene were 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 virus1, 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 and no unexpected cross-reactivity was observed for the organisms and virus listed. Results are shown in the table below.

Wet-testing cross-reactivity of 1copy™ COVID-19 qPCR 4plex Kit

Organism	Concentration	Results N Gene (#detected/ tested)	Results RdRp Gene (#detected/ tested)	Results E Gene (#detected/ tested)
Synthetic RNA of COVID-19 specific N gene	10^5 copies/ reaction	Positive signal (5/5)	Not detected (0/5)	Not detected (0/5)
Synthetic RNA of COVID-19 specific RdRp gene	10^5 copies/ reaction	Not detected (0/5)	Positive signal(5/5)	Not detected (0/5)
Synthetic RNA of beta-coronavirus specific E gene	10^5 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Positive signal(5/5)
Influenza A virus (H3N2) (Ref. KBPV_VR_32)	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)
Influenza A virus (H1N1) (Ref. KBPV_VR_33)	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)
Parainfluenzavirus 1	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)
Parainfluenzavirus 2	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)
Rhinovirus 14	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)
Enterovirus 71	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)
Escherichia coli (Ref. 25922)	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)
Human total RNA	10^6 copies/ reaction	Not detected (0/5)	Not detected (0/5)	Not detected (0/5)

13.4 Clinical evaluation

Performance of the 1copy™ COVID-19 qPCR 4plex Kit was evaluated using clinical nasopharyngeal and oropharyngeal swab specimens that were previously tested with an FDA EUA authorized SARS-CoV-2 molecular test.
A total of 12 positive and negative samples were used for the upper respiratory tract specimen of 6 positive and 6 negative samples were used. A total of 24 samples were used for six positive samples, including the original sample and the diluted sample diluted with 1/10, $10/10^2$ and $1/10^3$.

For the positive clinical samples, the positive percent agreement (PPA) between the 1copy™ COVID-19 qPCR 4plex Kit and the comparator assay was 100% (24/24). The Ct range for the E, N and RdRp targets used in the 1copy™ COVID-19 qPCR 4plex Kit for the 24 positive clinical samples was 15.48 - 33.28, 15.35 - 32.29 and 15.83 - 33.44 respectively. For the 6 clinical negative samples that were evaluated, 6/6 tested negative (100% NPA) using the 1copy™ COVID-19 qPCR 4plex Kit when run on the CFX96™ Real-Time PCR Detection system.

14. References

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8. Centers for Disease Control and Prevention.
Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primers and Probes. https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html
Accessed June 6, 2020.

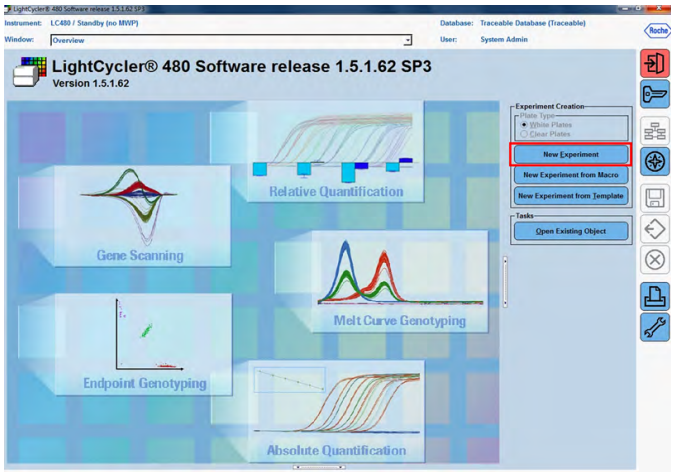
15. Glossary of Symbols

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
Σ	Contains sufficient for tests
	Use By Date
	Manufacturer
	Consult instructions for use
	Temperature limitation
	Caution

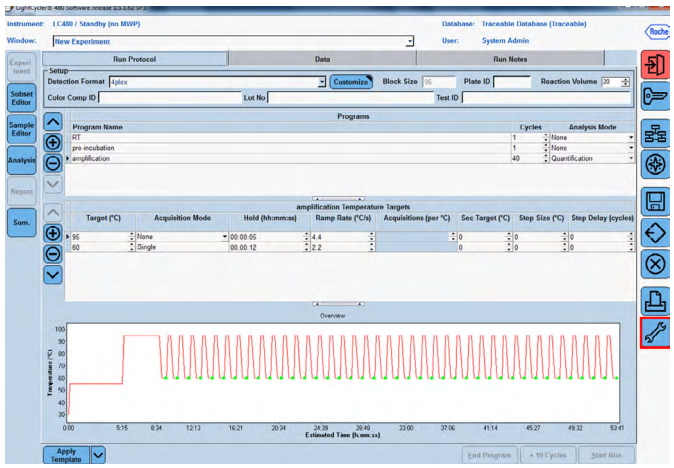
Appendix. Software Setting

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

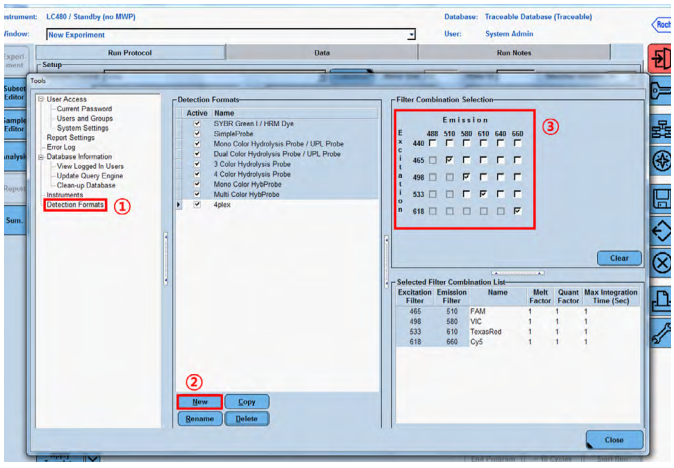
1.1 Run software and click “New Experiment”.



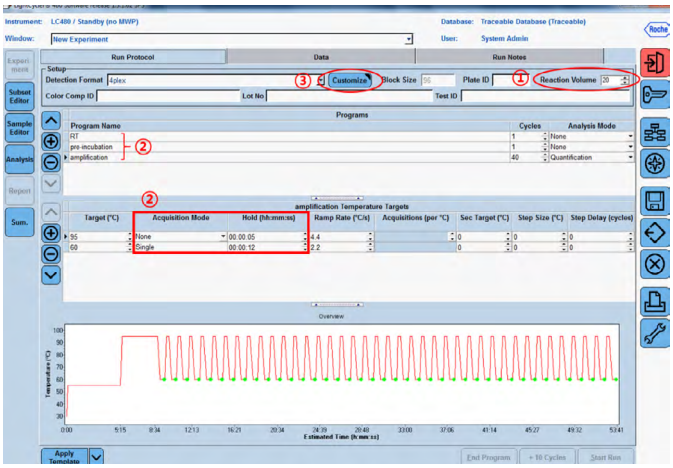
1.2 Click “Open Tools”.



1.3 Click “Detection Formats” and add new Detection Formats. Select FAM (465-510), VIC (498-580), Texas Red (533-610) and Cy5 (618-660).



1.4 Enter the reaction volume as 20 µℓ and modify PCR reaction conditions as below and click “Customize”.

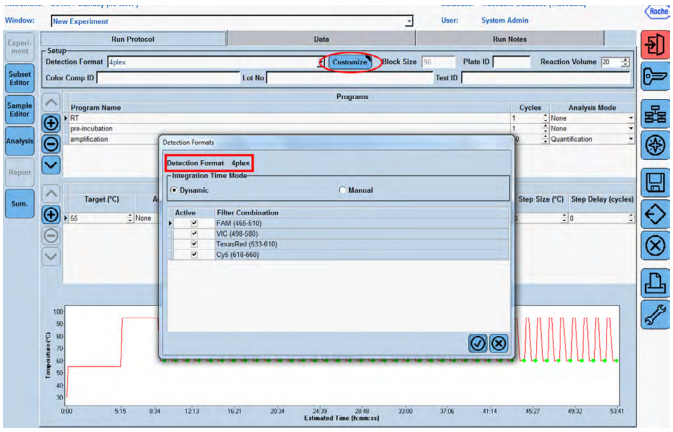


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

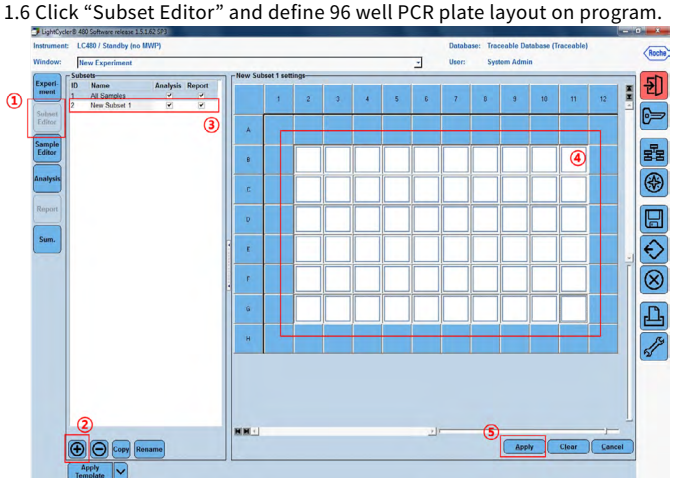
* Measure florescence at 60°C

* Time taken to run each PCR cycle may vary depending on the instrument used

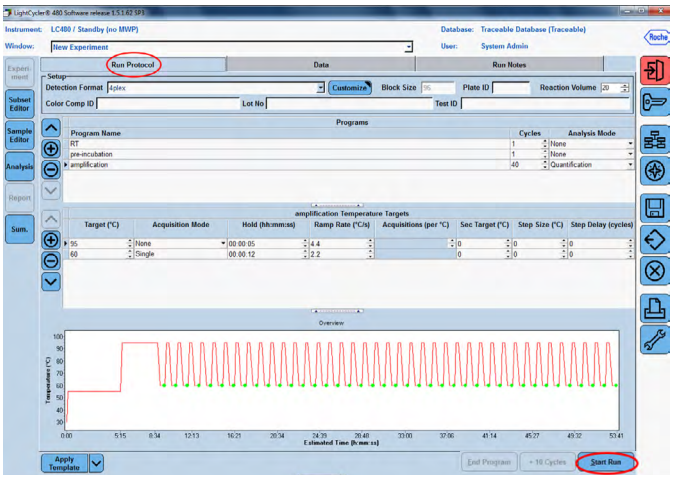
1.5 Select Detection Formats added in 1.3.



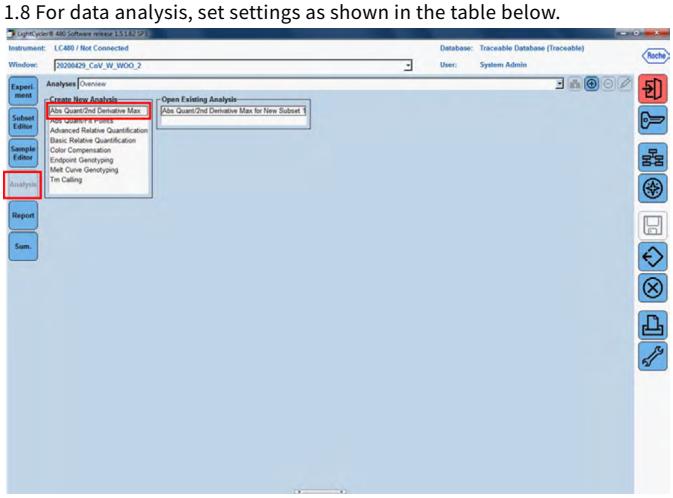
1.6 Click “Subset Editor” and define 96 well PCR plate layout on program.



1.7 Click “Run Protocol” on the above menu bar and then “Start Run”.



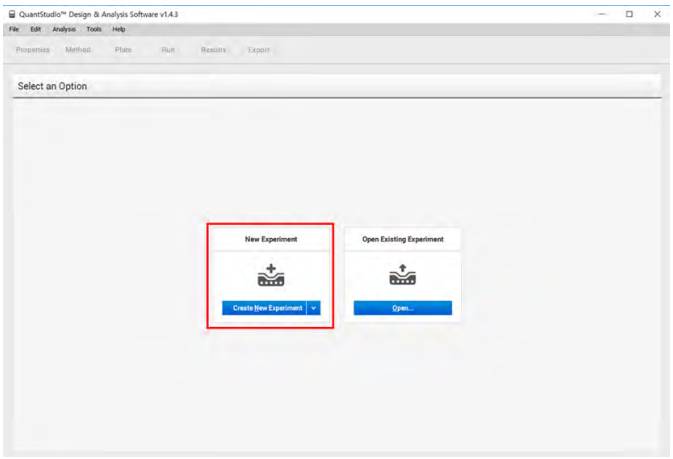
1.8 For data analysis, set settings as shown in the table below.



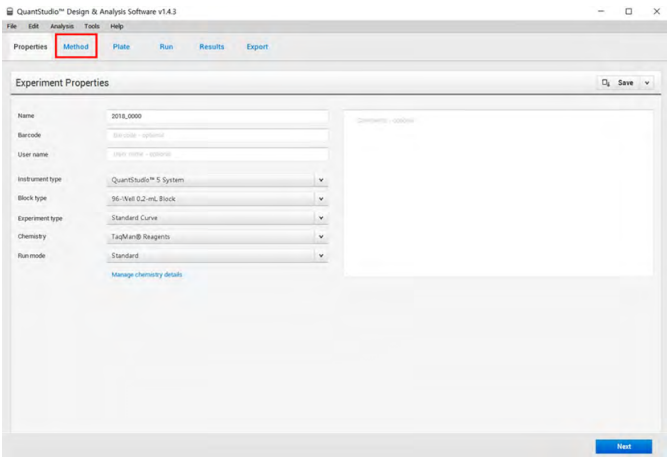
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

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

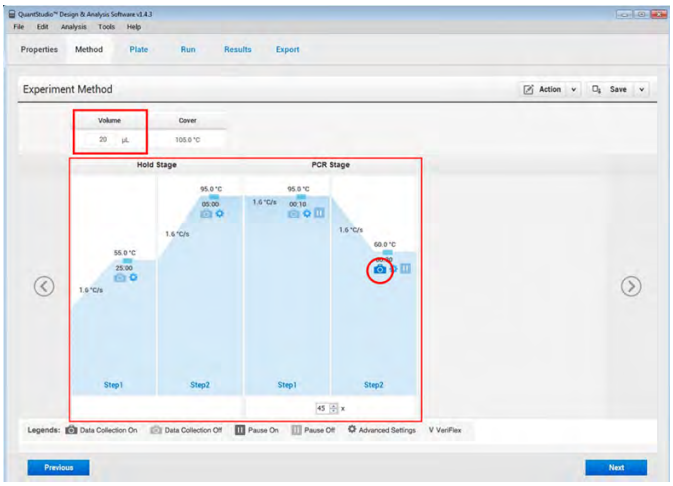
2.1 Run software and click “Create New Experiment” of “New Experiment”.



2.2 Click “Method” on the menu bar.



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

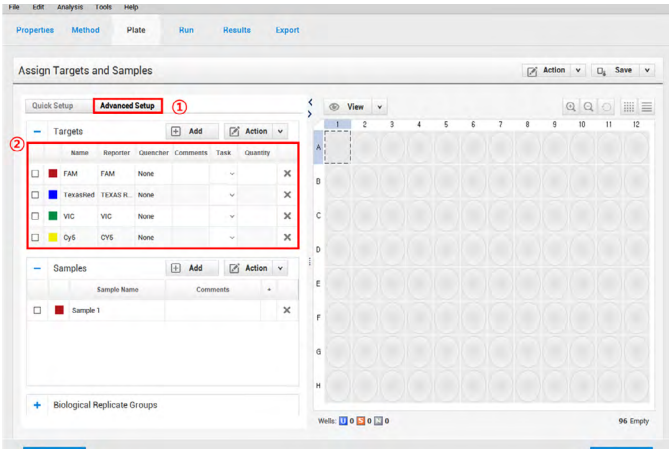


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

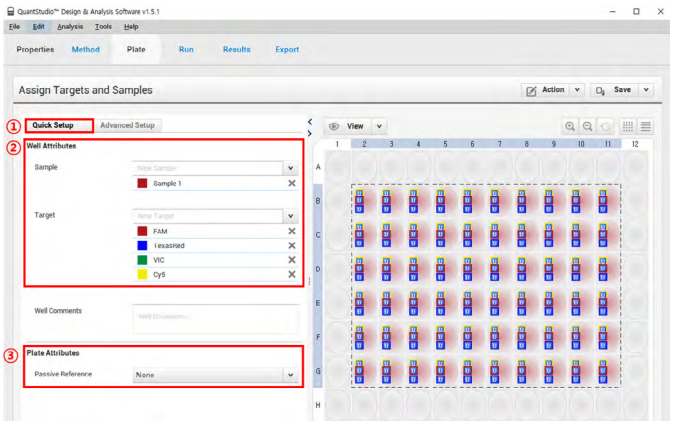
* Measure florescence at 60°C

* Time taken to run each PCR cycle may vary depending on the instrument used

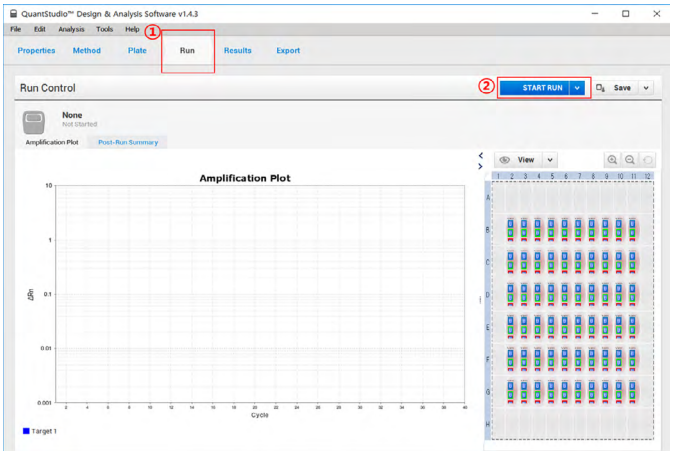
2.4 Click “Plate” on the menu bar and select “FAM” for Target1, “Texas Red” for Target2, “VIC” for Target3, and “Cy5” for Target4 in “Advanced Setup”.



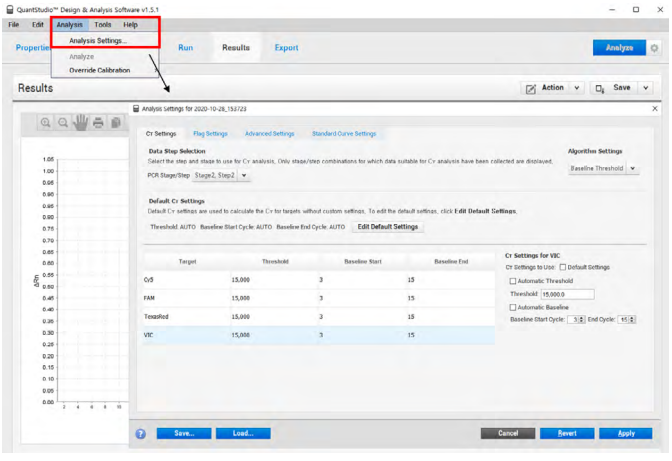
2.5 Click “Quick Setup” next to “Advanced Setup” and define 96 well PCR plate layout on program. Also, check the “Passive Reference : None”.



2.6 Click “Run ” on the menu bar and then “Start Run”.



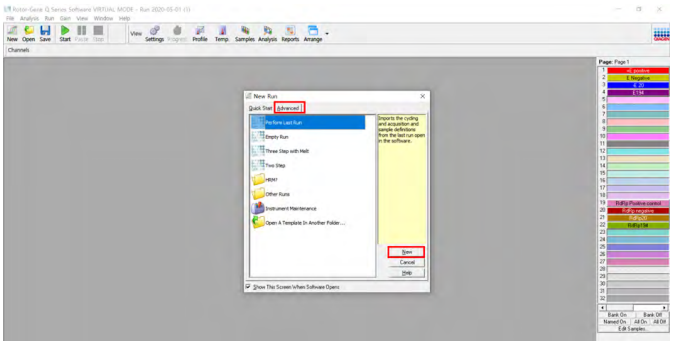
2.7 For data analysis, set settings as shown in the table below.



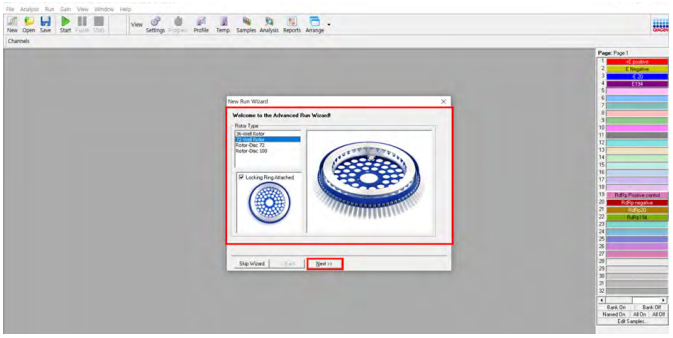
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

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

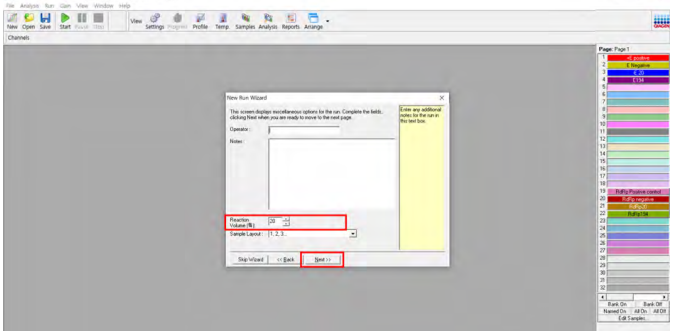
3.1 Run software and click “Advanced” and click “New”.



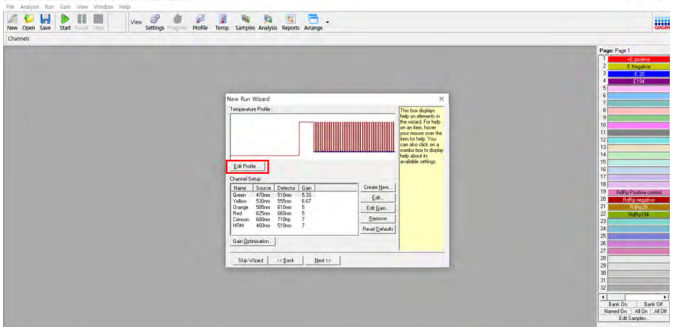
3.2 Check the rotor type and Click “Next”.



3.3 Enter the reaction volume as 20 µℓ and click “Next”.



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

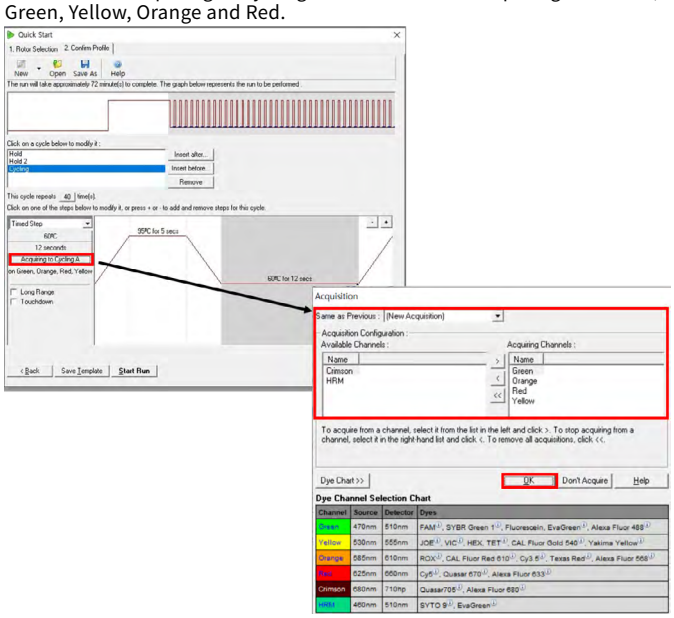


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

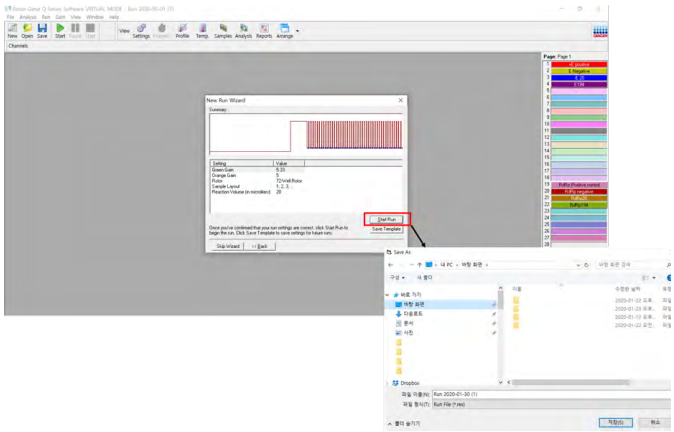
* Measure florescence at 60°C

* Time taken to run each PCR cycle may vary depending on the instrument used

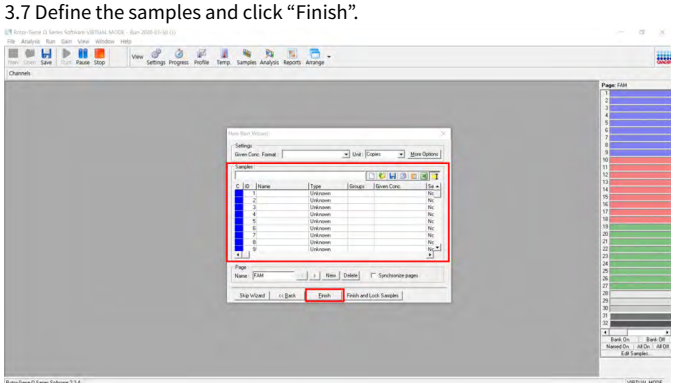
3.5 Click the “Acquiring to Cycling A” and check the “Acquiring channels”, Green, Yellow, Orange and Red.



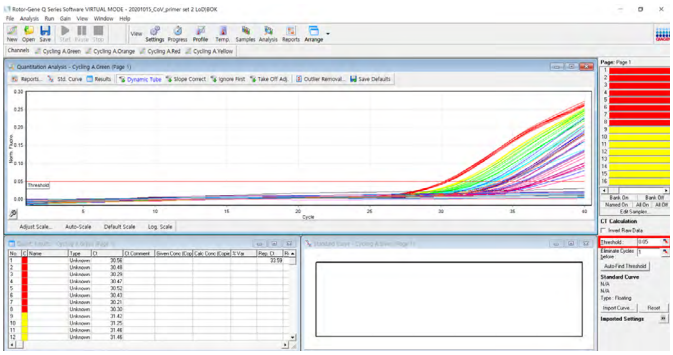
3.6 Click “Start Run” and save the file.



3.7 Define the samples and click “Finish”.



3.8 For data analysis, set settings as shown in the table below.

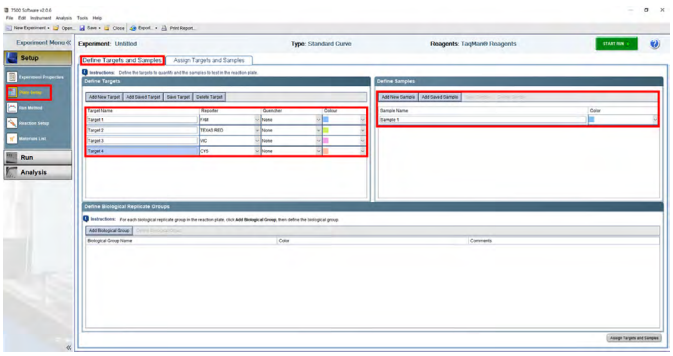


All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

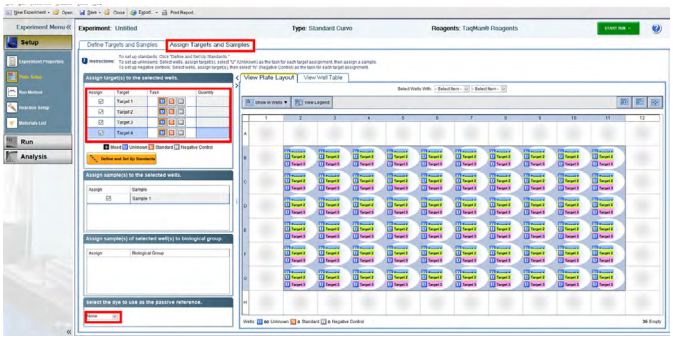
4. Applied Biosystems 7500 Real-Time PCR Instrument system (Thermo Fisher Scientific, Product No. 4345241)

4.1 Run software and click “Advanced setup”.

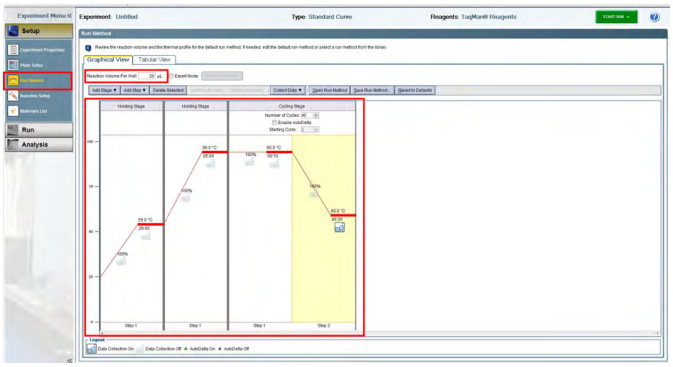
4.2 Click “Plate setup” and select “FAM” for Target1, ‘Texas Red” for Target2, “VIC” for Target3, and “Cy5” for Target4 in “Define Targets and Samples”



4.3 Click “Assign Targets and Samples” and define 96 well PCR plate layout on program. Also, select “None” in the “Select the dye to use as the passive reference”.



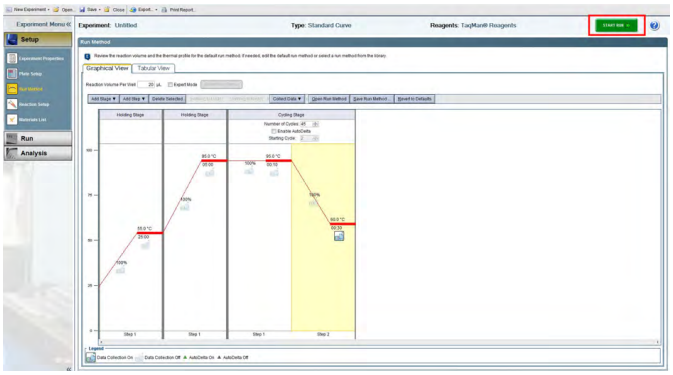
4.4 Click “Run Method” and enter the reaction volume as 20 µℓ and modify PCR reaction conditions as below.



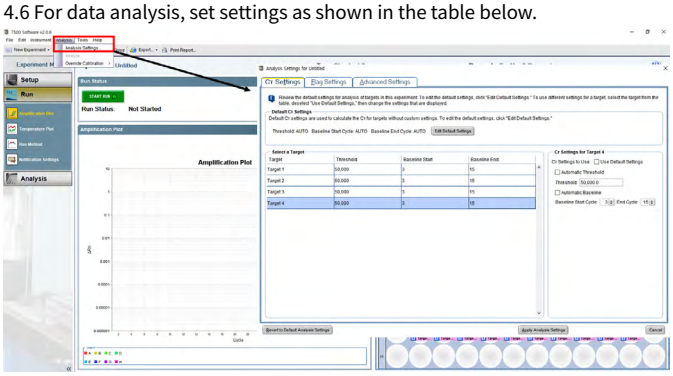
Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	28 sec	

* Measure florescence at 60°C
* For ABI 7500, set the amplification time for 60°C as 28 sec.
* Time taken to run each PCR cycle may vary depending on the instrument used

4.5 Click “Start Run”.



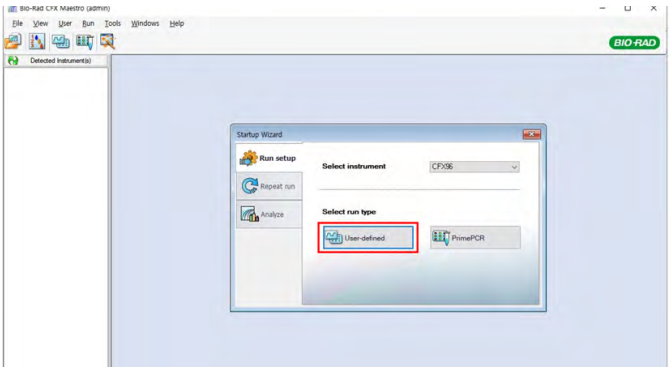
4.6 For data analysis, set settings as shown in the table below.



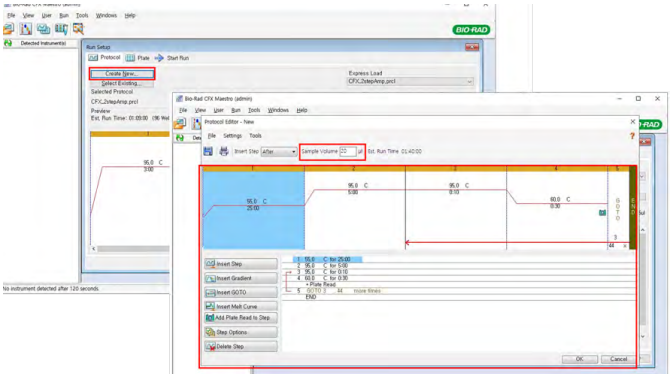
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

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

5.1 Run software and click “User-defined”.



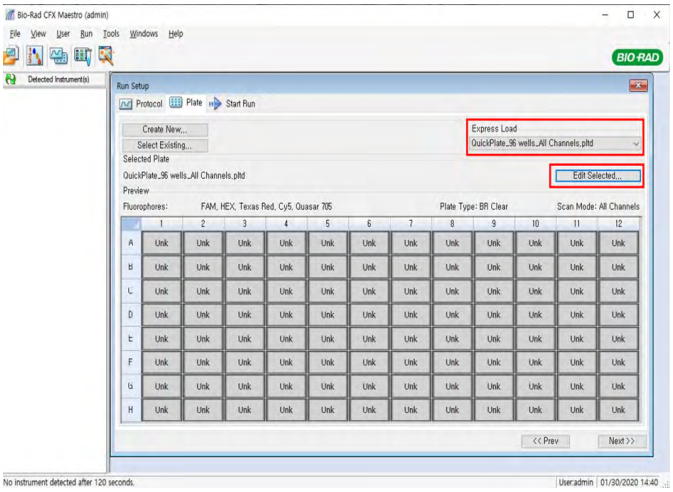
5.2 Click “Create New” and enter the reaction volume as 20 µℓ and modify PCR reaction conditions as below.



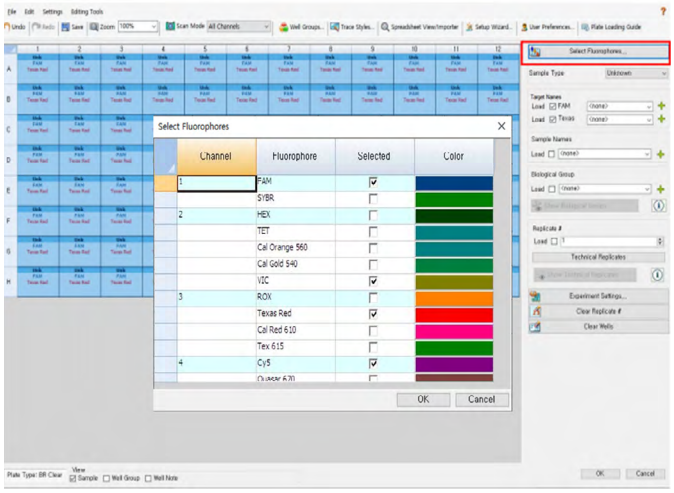
Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

* Measure florescence at 60°C
* Time taken to run each PCR cycle may vary depending on the instrument used

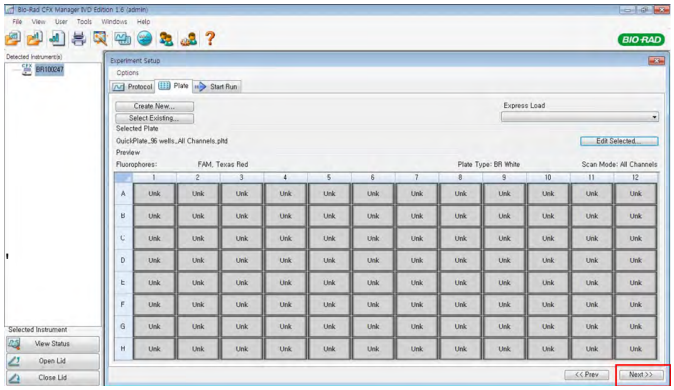
5.3 Click “Plate” and check the “Express Load : QuickPlate_96 wells_All Channels.pltd” and click “Edit selected”.

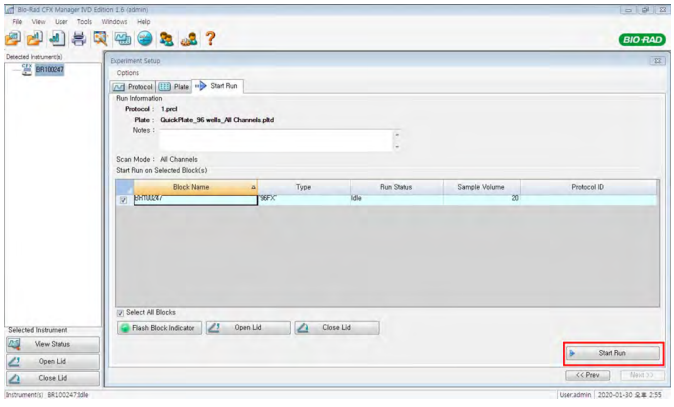


5.4 Click “Select Fluorophores” and check FAM, Texas Red, VIC and Cy5. Also, define 96 well PCR plate layout on program.

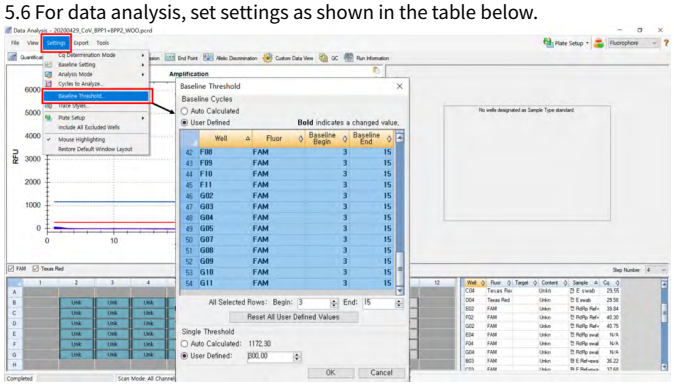


5.5 Click “Next” and click “Start Run”.





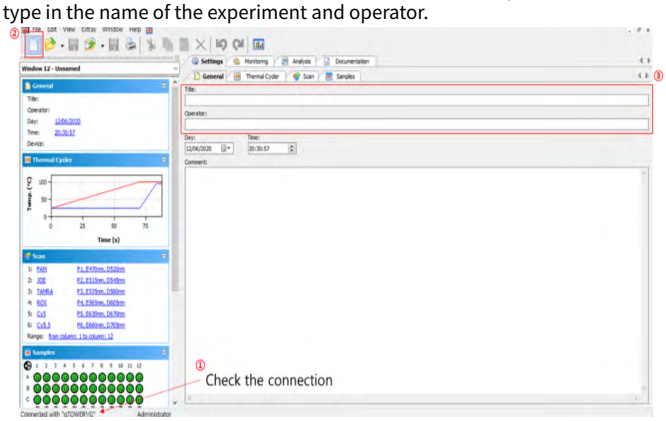
5.6 For data analysis, set settings as shown in the table below.



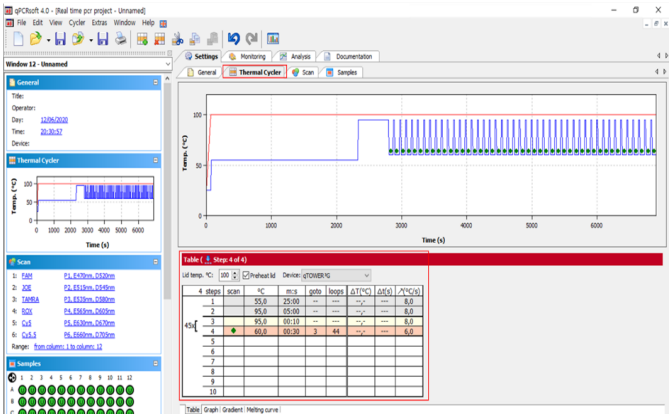
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

6. qTOWER3 Real-Time PCR Thermal Cycler (Analytik-jena, Product No. 844-00553-2)

6.1 Run software and check the connection. Click “New Experiment” and type in the name of the experiment and operator.



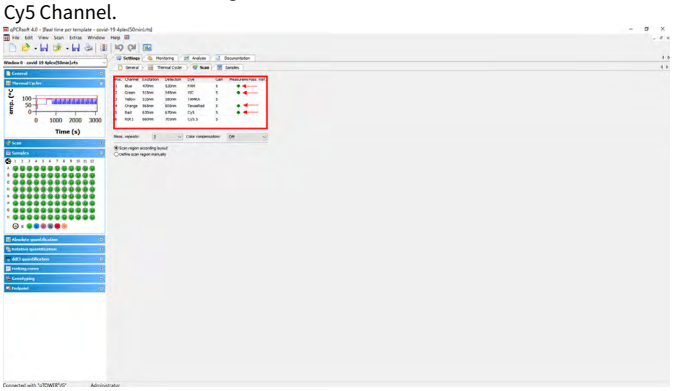
6.2 Click the Settings – Thermal Cycler and modify PCR reaction conditions as below.



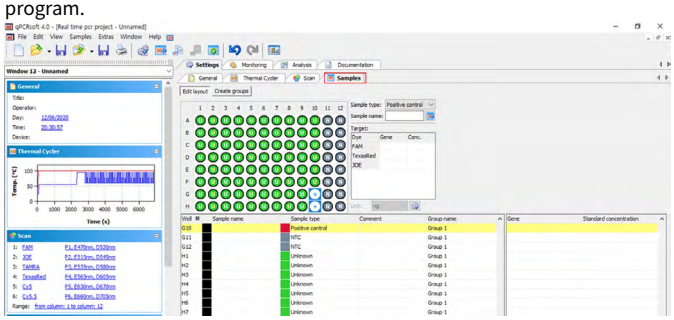
Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

* Measure florescence at 60°C
* Time taken to run each PCR cycle may vary depending on the instrument used

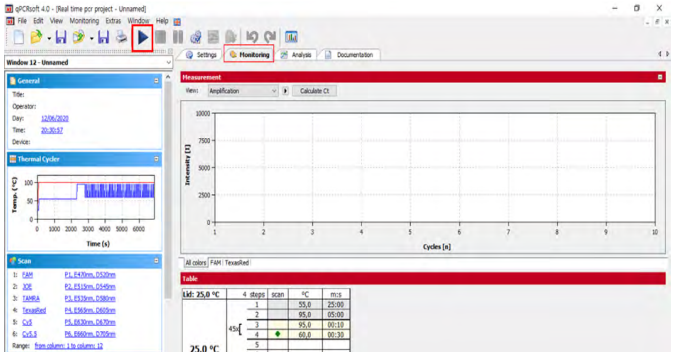
6.3 Click the menu “Settings” – “Scan” and Select FAM, Texas Red, VIC and Cy5 Channel.



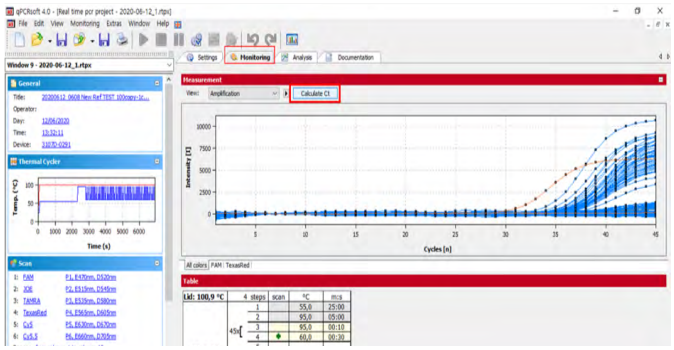
6.4 Click “Settings” – “Samples” and define 96 well PCR plate layout on program.



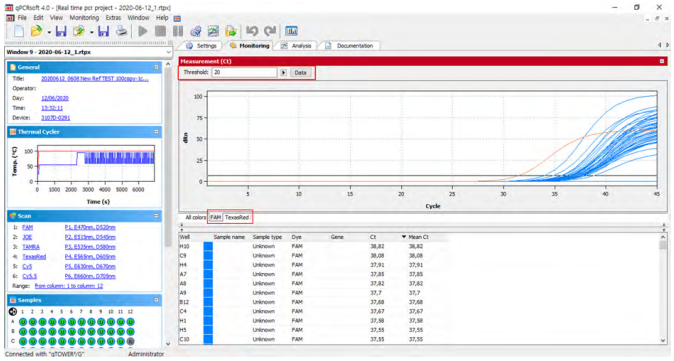
6.5 Click the menu – “Monitoring” and Click “Start Run” button.(▶)



6.6 After finish running, click the menu “Monitoring” and click “Calculate Ct”.



6.7 Check the Threshold, FAM : 7, Texas Red : 7, VIC : 7, and Cy5 : 7.
The Ct value changes automatically.



	Threshold						Baseline	
Target	CFX96	7500	Quantstudio5	qTOWER3	Rotor-Gene Q	LC480	Begin	End
FAM Texas Red VIC Cy5	500	50,000	15,000	7	0.05	Abs Quant/2nd Derivative Max	3	15

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