



## Instructions for Use

Rev. Date : April, 14th, 2021

Doc.no. DR-M23-6023-E-01

### 1. Description

1copy™ COVID-19 qPCR Triplex Kit provides reagents for real-time RT-PCR that specifically target the E (Envelope) gene and the N (Nucleocapsid protein) 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 Triplex 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 Triplex 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 Triplex 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, and VIC 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, N gene, IPC)	Brown (Amber tube)	100 µl
Control (E 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 ml or 0.2 ml PCR tubes or 96-well PCR plates specified in PCR instrument manufacturer's instructions
- 1.5 ml micro tubes
- Sealing film
- Ice or cooling/cold block
- Microliter pipettes (1~10 µl, 10~100 µl, 100~1000 µl)
- Mini centrifuge (0.2 ml/0.5 ml tubes, 10,000 rpm) or Benchtop centrifuge (1.5 ml microcentrifuge and 96 well plate centrifuge) with rotor for 0.2 ml/0.5 ml reaction tubes (capable of attaining 10,000 rpm)
- Vortex mixer
- Sample collection and sample preservation buffer (Puritan UniTranz-RT 3 ml 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 Triplex 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 Triplex 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 mL 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

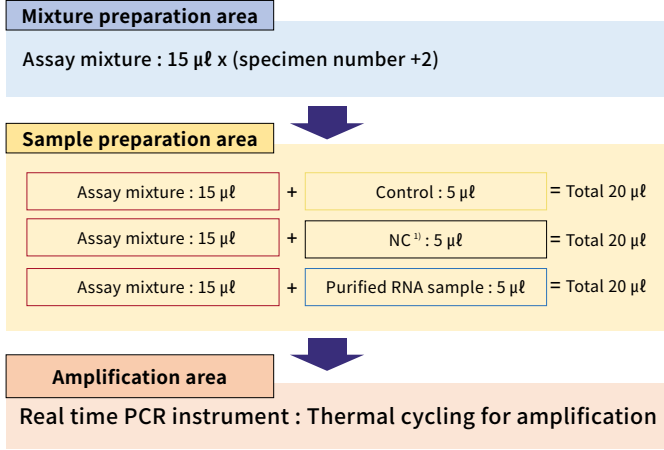
\* Validated Kit for extraction of nucleic acids

- 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



<sup>1)</sup> 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 Triplex 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

\* 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
N gene	Texas Red			Texas Red	Orange	Cy5
IPC	VIC			JOE	Yellow	VIC

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

FAM, Texas Red and VIC 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, 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  $\leq 40$ .

Ct value	Result
$\leq 40$	Detected (+)
$> 40$ or N/A	Not Detected (-)

Ct values above 40 for FAM, Texas Red and VIC 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.

If carry-over contaminations occur frequently in the range of Ct values from 35 to 40, the cut-off value can be manually adjusted to reduce the probability of false-positives and improve assay performance.

※ Set threshold values and baseline

All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC	500	50,000	15,000	20	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	Texas Red	VIC	FAM	Texas Red	VIC	
+			-			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  $\leq 40$  = Detected (+), Ct  $> 40$  = Not Detected (-)

### 11.3 Patient specimen interpretation

E gene assay (FAM)	N gene assay (Texas Red)	IPC (VIC)	Interpretation
+		+/-	Positive for SARS-CoV-2
-		+	Negative for SARS-CoV-2
+	-	+/-	Inconclusive** Sample must be retested
-	+	+/-	
-		-	Invalid Result* Repeat extraction and RT-PCR, If result from repeated run is invalid, consider collecting new samples

\* Invalid result due to potential sampling error or inhibition.

\*\*Inconclusive: A positive result in single target (E gene or N gene) may be suggestive of

- 1) a sample at concentrations near or below the limit of detection of the test,
- 2) a mutation in the 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.

Sample should be retested.

※ Note: Ct  $\leq 40$  = 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 Triplex 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 Triplex Kit may give in inaccurate results.
- As with any molecular test, mutations within the target regions of 1copy™ COVID-19 qPCR Triplex 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 Triplex 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 Triplex Kit. The LoD of 1copy™ COVID-19 qPCR Triplex 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. 0505-0129, stock concentration 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup> 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 19/20 replicates are positive for each assay target.

The LoD for 1copy™ COVID-19 qPCR Triplex 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

### 13.2 Inclusivity

The inclusivity of 1copy™ COVID-19 qPCR Triplex Kit was evaluated using *in silico* analysis of the assay primers and probes in relation to 3437 SARS-CoV-2 sequences available in the GISAID gene database released on July 5, 2020 for two targets, E and N.

For the E gene, 1copy™ COVID-19 qPCR Triplex Kit had 100% match to all sequences with the exception of 9 sequences that had a single mismatch. For the N gene, 1copy™ COVID-19 qPCR Triplex Kit had 100% match to all sequences with the exception of 10 sequences that had a single mismatch. None of these mismatches found for both targets are predicted to negatively influence on the assay performance, given the location of the mutations in the primer and probe regions respectively for the two variants. 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
	<i>Chlamydia pneumoniae</i>
	<i>Haemophilus influenzae</i>
	<i>Legionella pneumophila</i>
	<i>Mycobacterium tuberculosis</i>
	<i>Streptococcus pneumoniae</i>
	<i>Streptococcus pyogenes</i>
	<i>Bordetella pertussis</i>
	<i>Mycoplasma pneumoniae</i>
	<i>Pneumocystis jirovecii</i> (PJP)
	<i>Candida albicans</i>
	<i>Pseudomonas aeruginosa</i>
	<i>Staphylococcus epidermis</i>
	<i>Streptococcus salivarius</i>

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 N gene primers and probes in 1copy™ COVID-19 qPCR Triplex 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 Triplex 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 Triplex 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 N 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 Triplex Kit

Organism	Concentration	Results E Gene (#detected/tested)	Results N Gene (#detected/tested)
Synthetic RNA of SARS-CoV-2 specific E gene	10 <sup>5</sup> copies/mL	Detected (5/5)	Not detected (0/5)
Synthetic RNA of SARS-CoV-2 specific N gene	10 <sup>5</sup> copies/mL	Not detected (0/5)	Detected (5/5)
Influenza A virus (H3N2) (Ref. KBPV_VR_32)	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)
Influenza A virus (H1N1) (Ref. KBPV_VR_33)	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)
Parainfluenza virus 1	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)
Parainfluenza virus 2	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)
Rhinovirus 14	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)
Enterovirus 71	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)
<i>Escherichia coli</i> (Ref. 25922)	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)
Human total RNA	10 <sup>6</sup> copies/mL	Not detected (0/5)	Not detected (0/5)



### 13.4 Clinical evaluation

Performance of the 1copy™ COVID-19 qPCR Triplex Kit was evaluated using clinical nasopharyngeal and oropharyngeal swab specimens that were previously tested with an FDA EUA authorized SARS-CoV-2 molecular test.

For the positive clinical nasopharyngeal swab samples, the positive percent agreement (PPA) between the 1copy™ COVID-19 qPCR Triplex Kit and the comparator assay was 100% (30/30). The Ct range for the E and N targets used in the 1copy™ COVID-19 qPCR Triplex Kit for the 30 positive clinical samples was 20.46 – 32.25 and 20.14 – 32.69, respectively. For the 30 clinical negative samples that were evaluated, 30/30 tested negative (100% NPA) using the 1copy™ COVID-19 qPCR Triplex Kit when run on the CFX96™ Real-Time PCR Detection system. Qualitative results of the clinical evaluation are shown in the table below.

Nasopharyngeal swab samples		Authorized Assay - Comparator		
		Positive	Negative	Total
1copy™ COVID-19 qPCR Triplex Kit	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive percent agreement		100% (95%CI: 88.60% ~ 100%)		
Negative percent agreement		100% (95%CI: 88.60% ~ 100%)		












For the positive clinical oropharyngeal swab samples, the positive percent agreement (PPA) between the 1copy™ COVID-19 qPCR Triplex Kit and the comparator assay was 100% (30/30). The Ct range for the E and N targets used in the 1copy™ COVID-19 qPCR Triplex Kit for the 30 positive clinical samples was 18.93 – 36.57 and 18.67 – 35.47, respectively. For the 30 clinical negative samples that were evaluated, 30/30 tested negative (100% NPA) using the 1copy™ COVID-19 qPCR Triplex Kit when run on the CFX96™ Real-Time PCR Detection system. Qualitative results of the clinical evaluation are shown in the table below.

Oropharyngeal swab samples		Authorized Assay - Comparator		
		Positive	Negative	Total
1copy™ COVID-19 qPCR Triplex Kit	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60
Positive percent agreement		100% (95%CI: 88.60% ~ 100%)		
Negative percent agreement		100% (95%CI: 88.60% ~ 100%)		

## 14. References

- Centers for Disease Control and Prevention. <https://www.cdc.gov/coronavirus/2019-nCoV/index.html>. Accessed February 9, 2020.
- Centers for Disease Control and Prevention. Biosafety in Microbiological and Biomedical laboratories (refer to latest edition). <http://www.cdc.gov/biosafety/publications/>
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- World Health Organization. Laboratory Biosafety Manual. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.
- 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](https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2)
- 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>
- 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

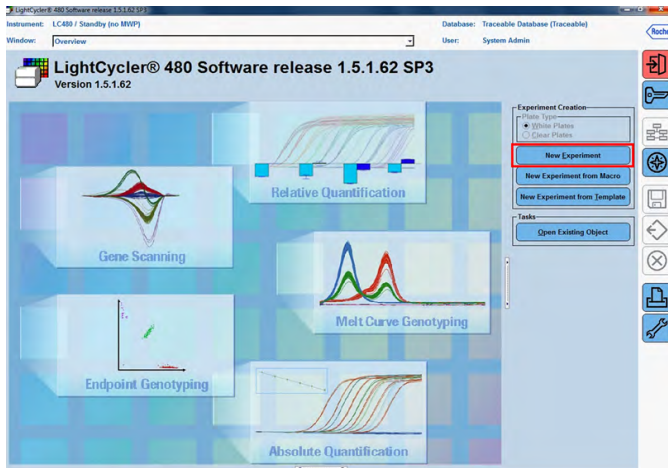
	Catalog Number
	This product fulfills the requirement for directive on <i>in vitro</i> diagnostic medical devices (Conformite Europeenne)
	In-Vitro-Diagnostic Medical Device
	Authorized representative in the European community
	Batch Code
	Contains sufficient for tests
	Use By Date
	Manufacturer
	Consult instructions for use
	Temperature limitation
	Caution

## Appendix. Software Setting

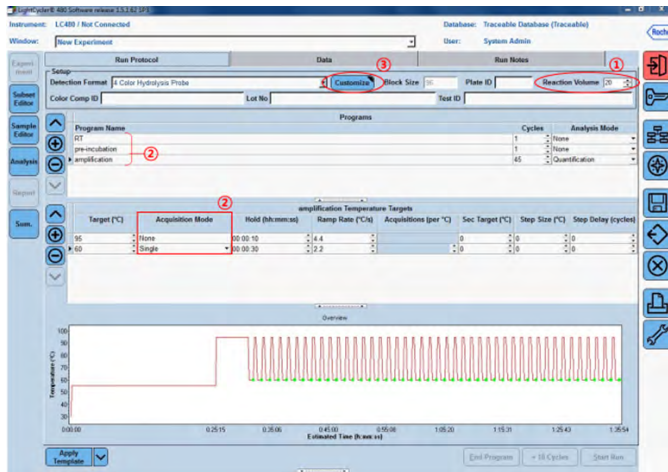
### 1) Light Cycler 480

(Roche, Product No. 05015278001)

① Run software and click “New Experiment”.



② Enter the reaction volume as 20 µl and modify PCR reaction conditions as below.

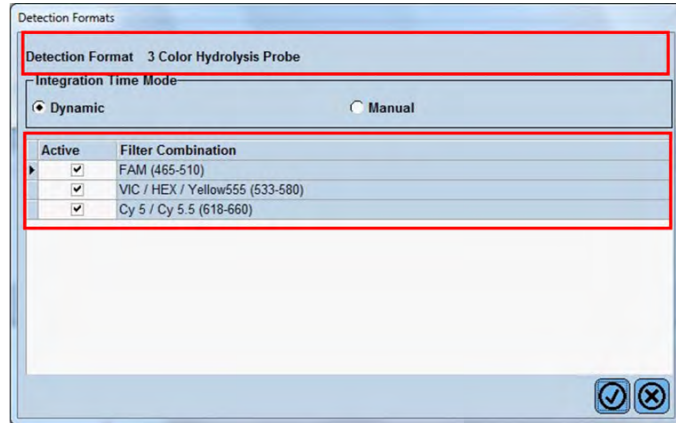
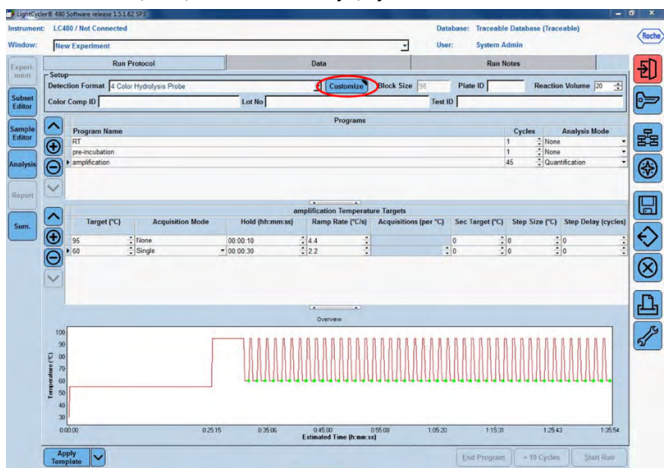


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

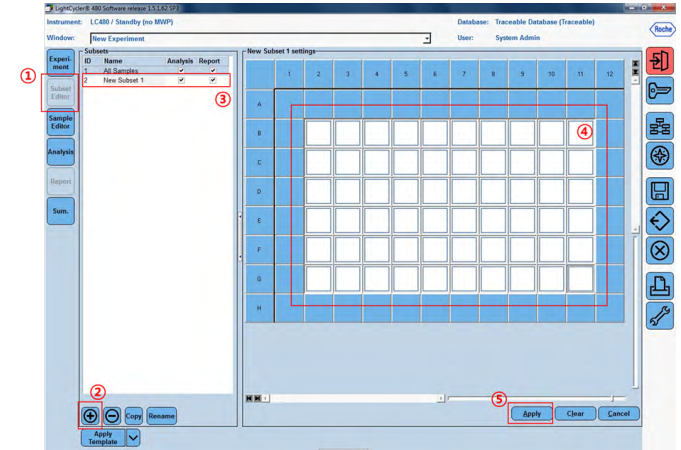
\* Measure fluorescence at 60°C

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

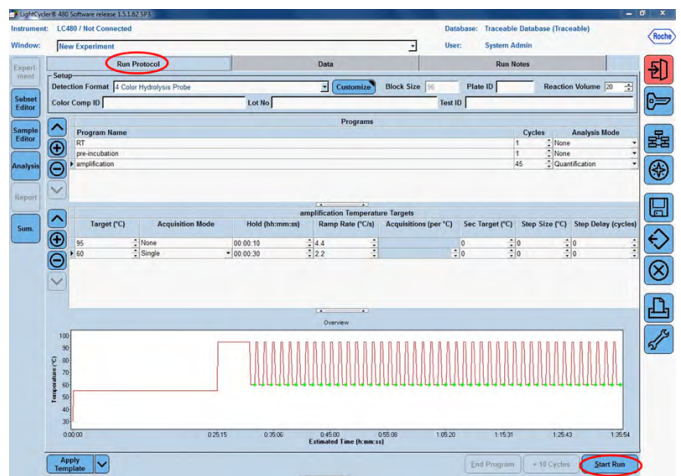
③ Click “Customize” and select 3 Color Hydrolysis probe “FAM” & “VIC/HEX/Yellow555” & “Cy5/Cy5.5”.



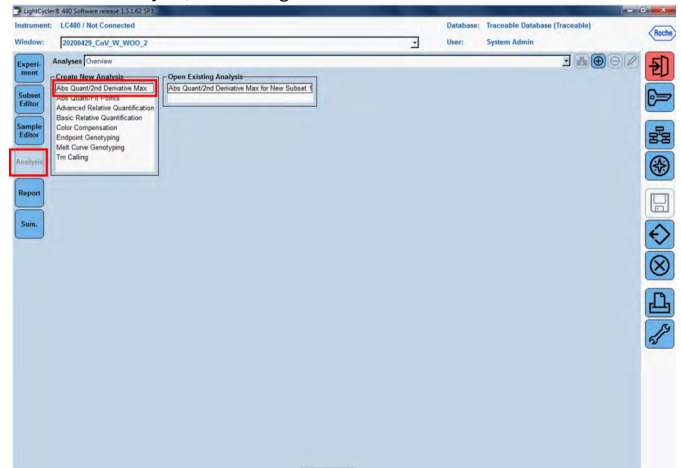
④ Click “Subset Editor” and define 96 well PCR plate layout on program.



⑤ Click “Run Protocol” on the above menu bar and then “Start Run”.



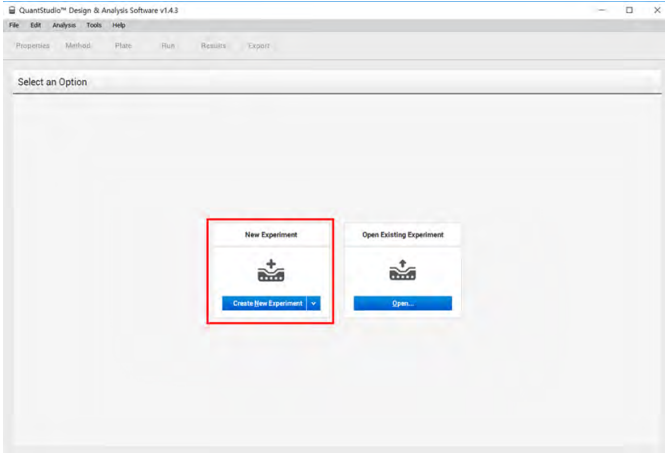
⑥ For data analysis, set settings as shown in the table below.



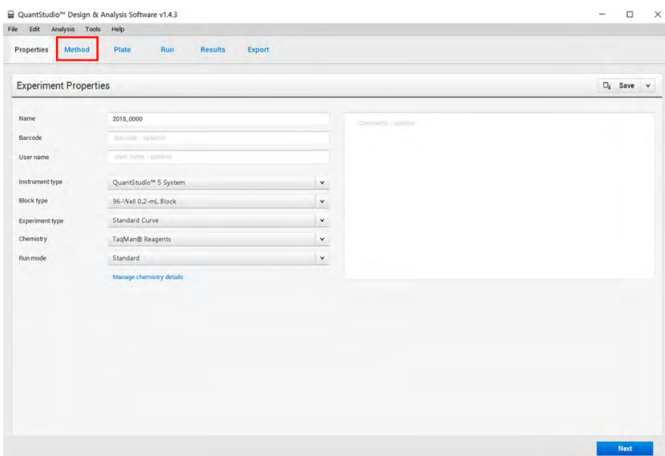
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC	500	50,000	15,000	20	0.05	Abs Quant/2nd Derivative Max	3	15

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	

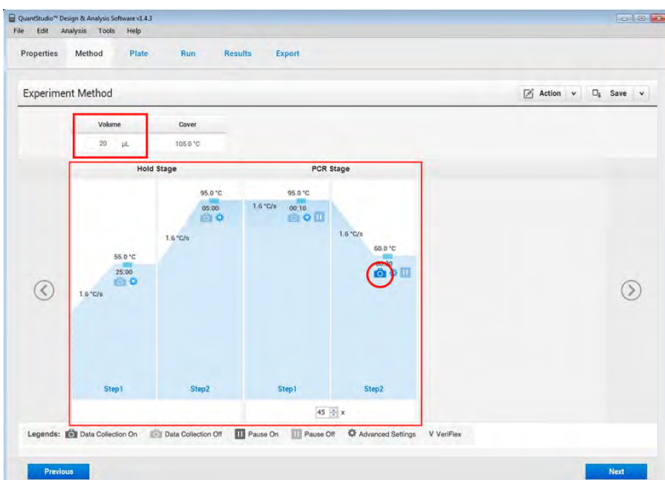
- 2) Applied Biosystems Quantstudio5  
(Thermo Fisher Scientific, Product No. A28134)  
① Run software and click “Create New Experiment” of “New Experiment”.



- ② Click “Method” on the menu bar.

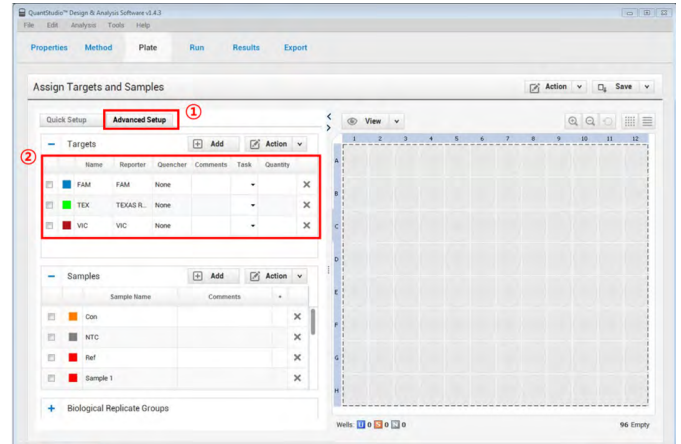


- ③ Enter the reaction volume as 20 µl and modify PCR reaction conditions as below.

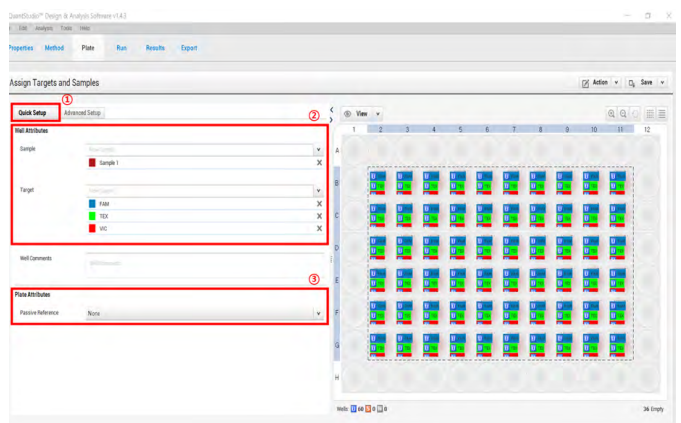


- \* Measure fluorescence at 60°C  
\* Time taken to run each PCR cycle may vary depending on the instrument used

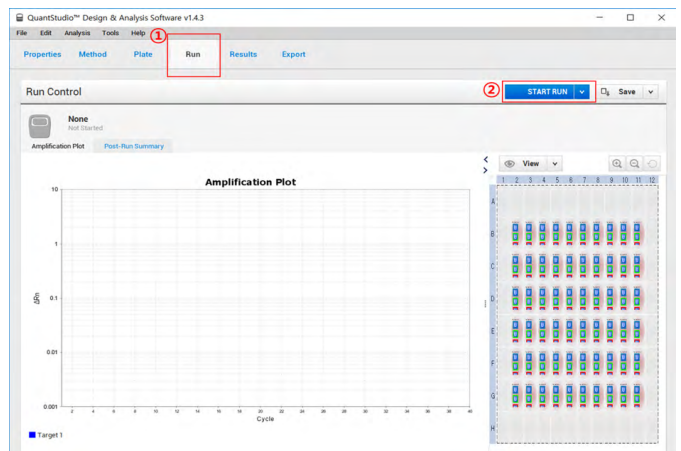
- ④ Click “Plate” on the menu bar and select “FAM” for Target1, “TEX Red” for Target2 and “VIC” for Target3 in “Advanced Setup”.



- ⑤ Click “Quick Setup” next to “Advanced Setup” and define 96 well PCR plate layout on program. Also, check the “Passive Reference : None”.

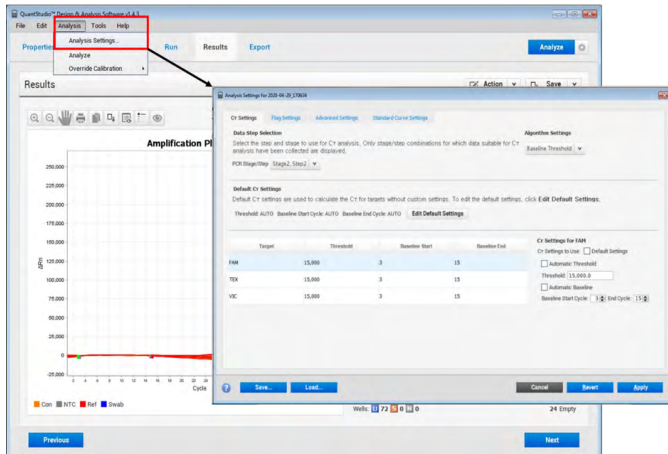


- ⑥ Click “Run ” on the menu bar and then “Start Run”.





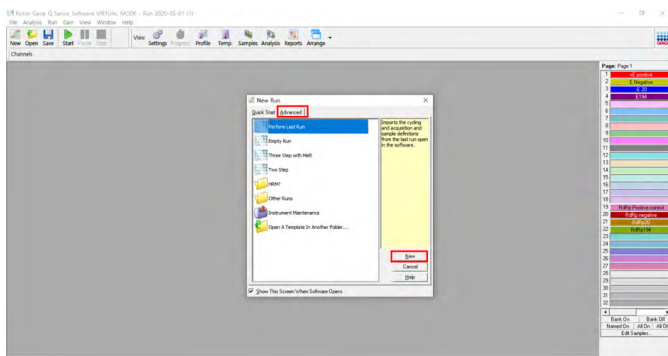
⑦ For data analysis, set settings as shown in the table below.



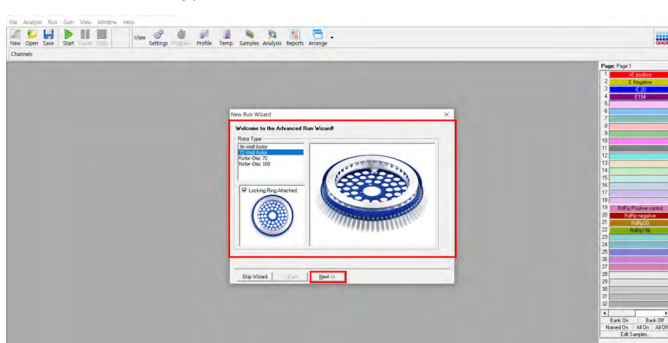
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC	500	50,000	15,000	20	0.05	Abs Quant/2nd Derivative Max	3	15

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

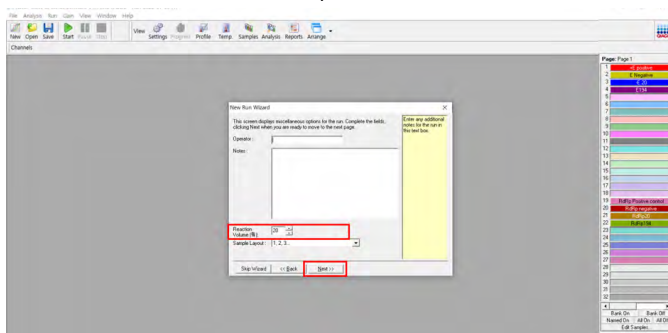
① Run software and click “Advanced” and click “New”.



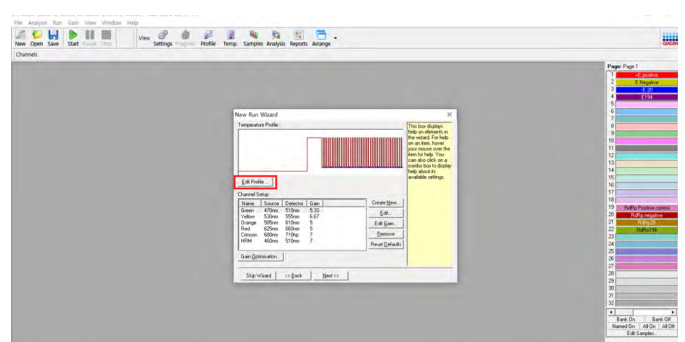
② Check the rotor type and Click “Next”.



③ Enter the reaction volume as 20 µl and click “Next”.



④ Click “Edit profile” and modify PCR reaction conditions as below.

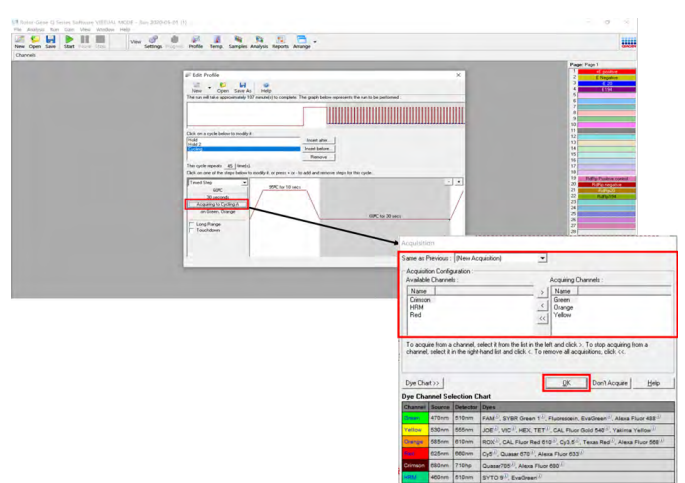


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

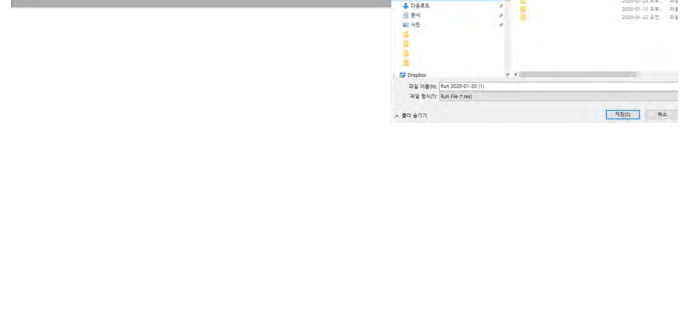
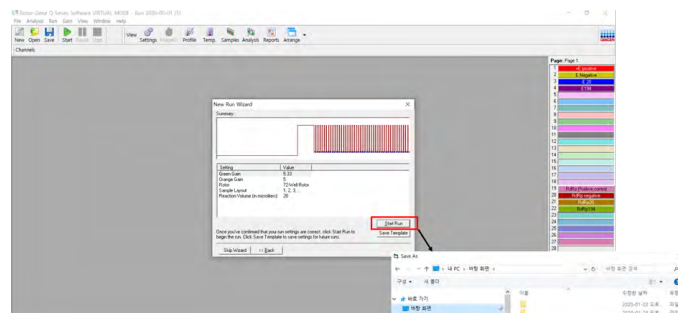
\* Measure fluorescence at 60°C

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

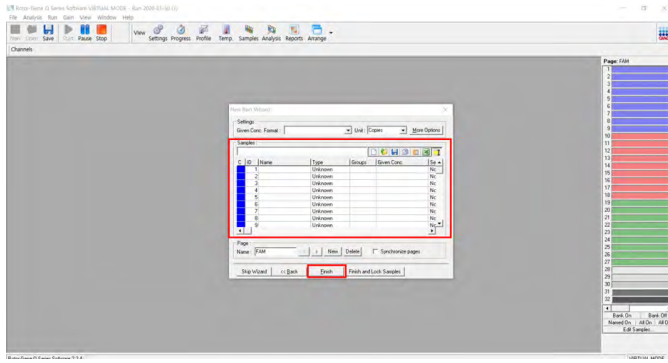
⑤ Click the “Acquiring to Cycling A” and check the “Acquiring channels”, Green, Yellow, and Orange.



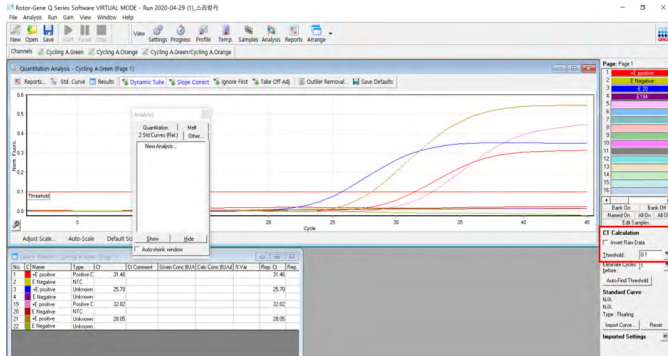
⑥ Click “Start Run” and save the file.



⑦ Define the samples and click “Finish”.



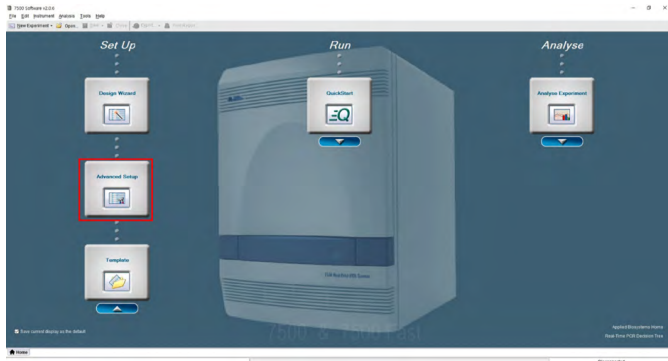
⑧ For data analysis, set settings as shown in the table below.



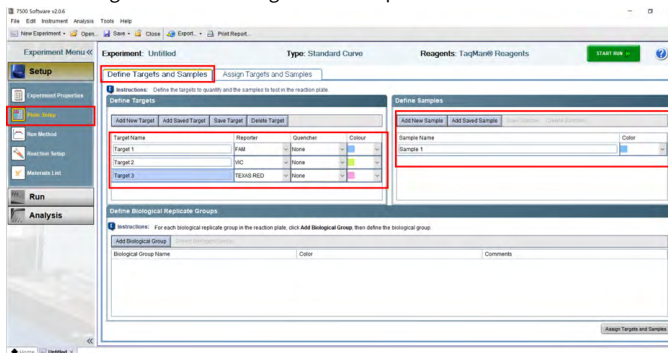
All Channel	Threshold					Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	
FAM						Abs	
Texas Red						Quant/2nd	
VIC	500	50,000	15,000	20	0.05	Derivative Max	
							Begin End
							3 15

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

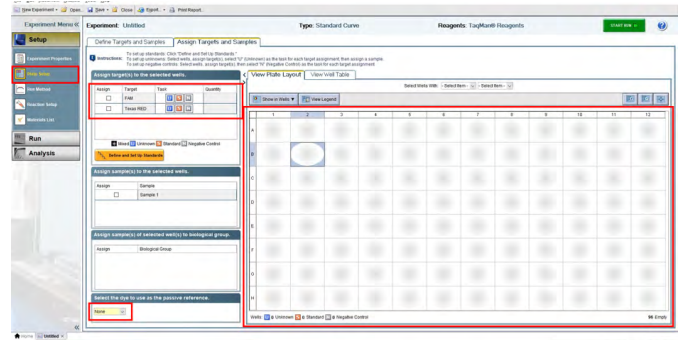
① Run software and click “Advanced setup”.



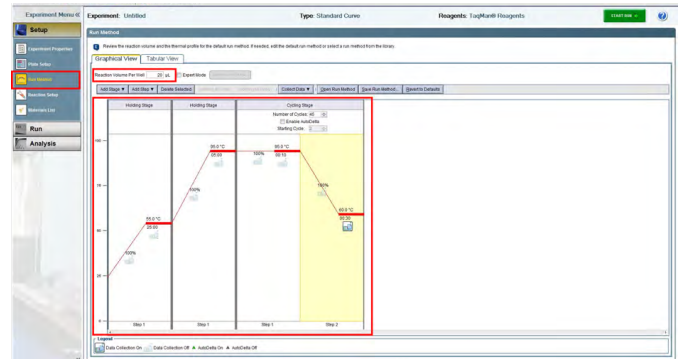
② Click “Plate setup” and select “FAM” for Target1, ‘VIC’ for Target2 and “TEX Red” for Target3 in “Define Targets and Samples”.



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

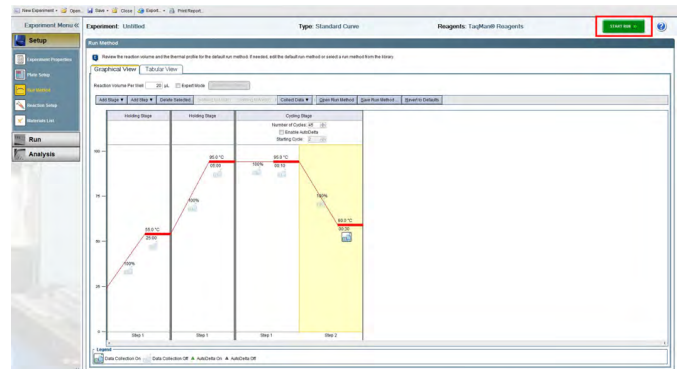


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

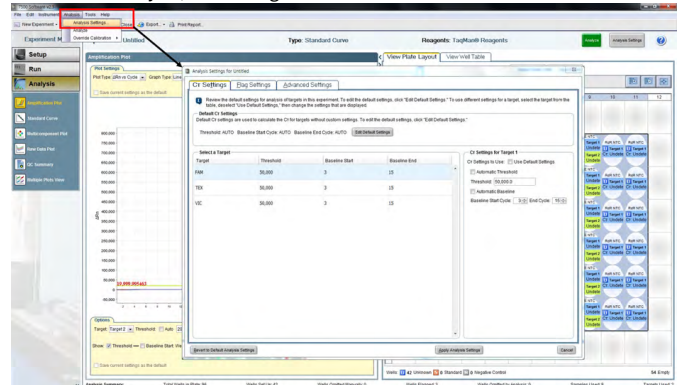
\* Measure fluorescence at 60°C

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

⑤ Click “Start Run”.



⑥ For data analysis, set settings as shown in the table below.

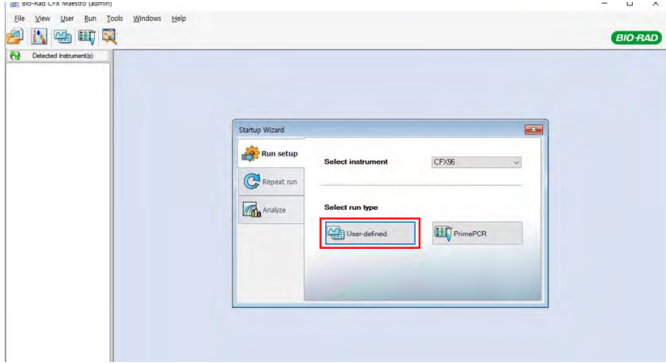




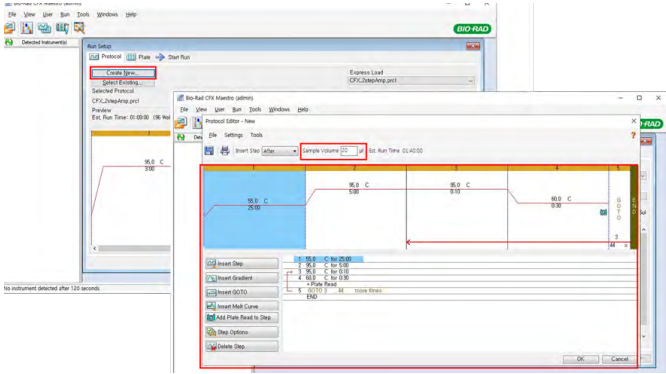
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC	500	50,000	15,000	20	0.05	Abs Quant/2nd Derivative Max	3	15

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

① Run software and click “User-defined”.



② Click “Create New” and enter the reaction volume as 20 µl and modify PCR reaction conditions as below.

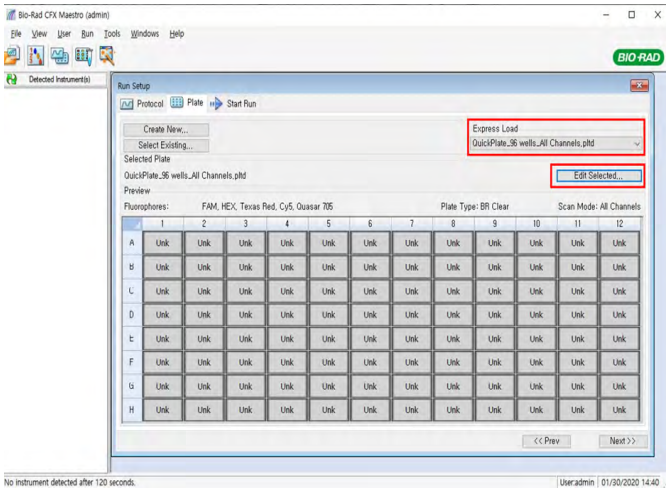


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

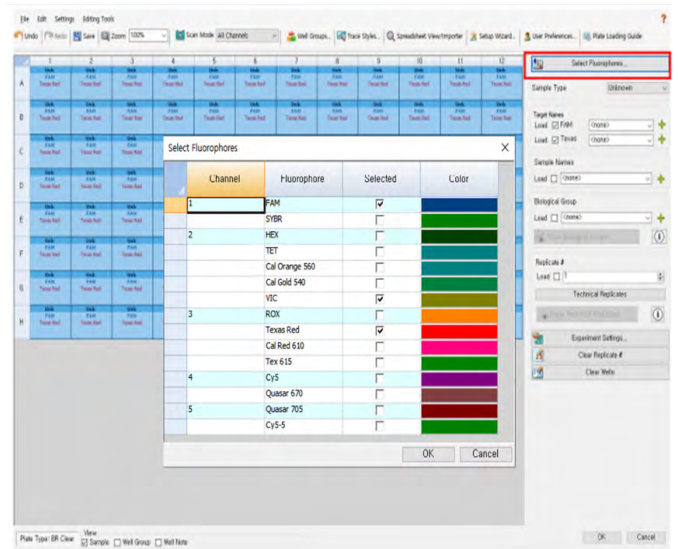
\* Measure florescence at 60°C

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

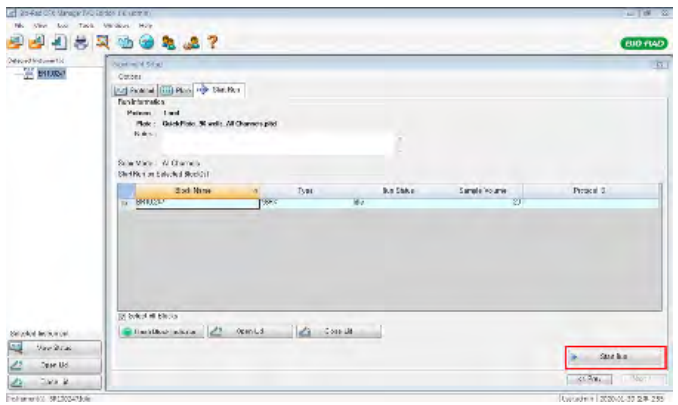
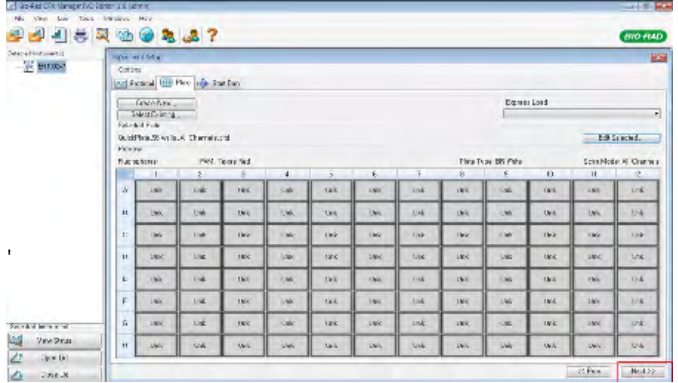
③ Click “Plate” and check the “Express Load : QuickPlate\_96 wells\_All Channels.pltd” and click “Edit selected”.



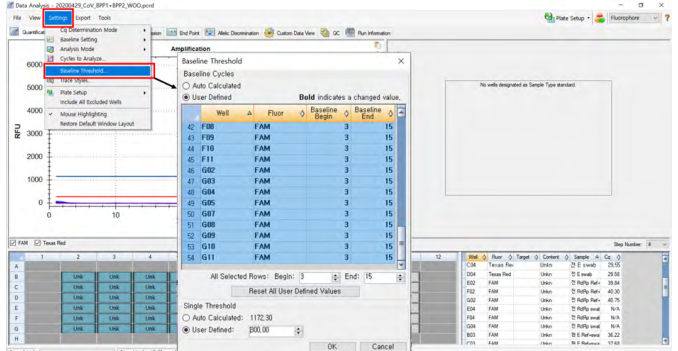
④ Click “Select Fluorophores” and check FAM, VIC and Texas Red. Also, define 96 well PCR plate layout on program.



⑤ Click “Next” and click “Start Run”.



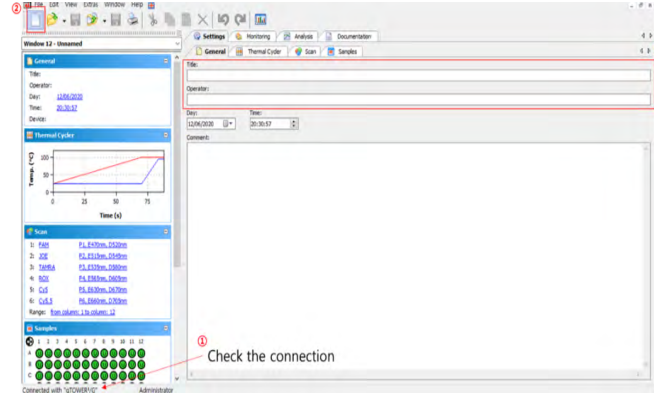
⑥ For data analysis, set settings as shown in the table below.



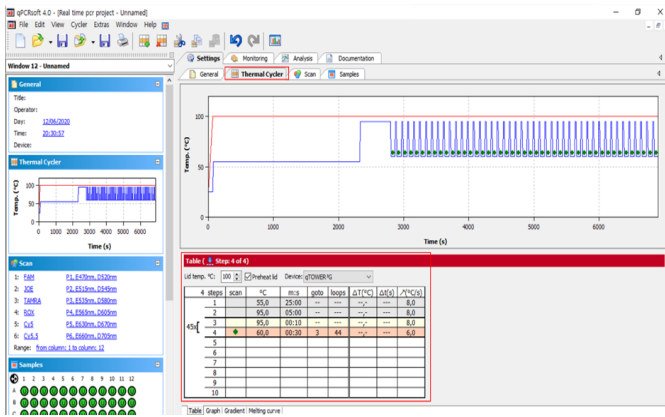
All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC	500	50,000	15,000	20	0.05	Abs Quant/2nd Derivative Max	3	15

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

① Run software and check the connection. Click “New Experiment” and type in the name of the experiment and operator.



② Click the Settings – Thermal Cycler and modify PCR reaction conditions as below.

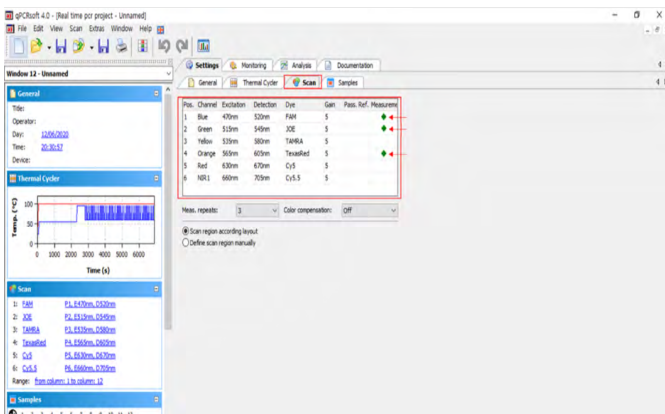


Step	Temperature	Time	Cycle
RT	55°C	5 min	1
Incubation	95°C	3 min	1
Amplification	95°C	5 sec	40
	60°C *	12 sec	

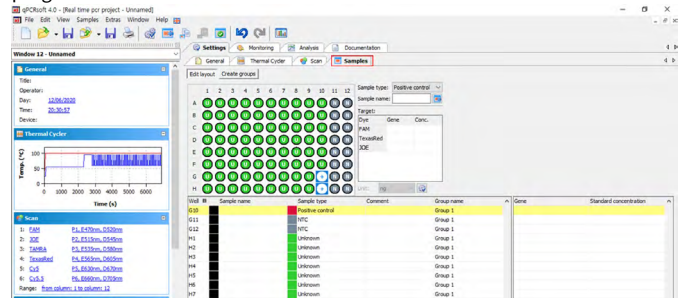
\* Measure florescence at 60°C

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

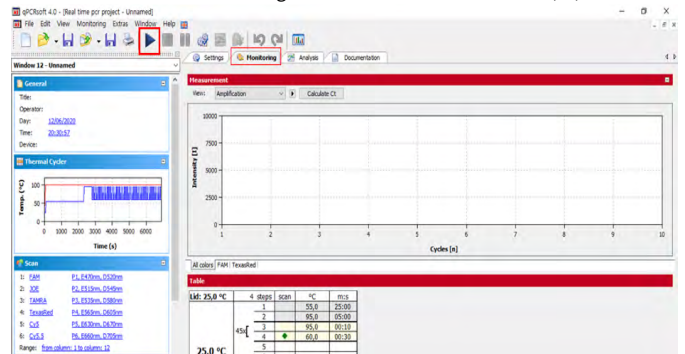
③ Click the menu “Settings”–“Scan” and Select FAM, JOE and Texas Red Channel.



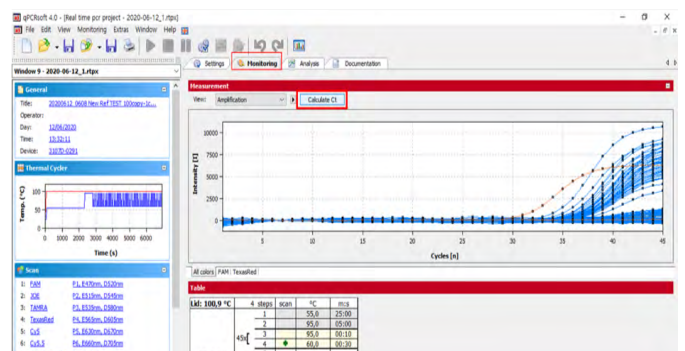
④ Click “Settings” – “Samples” and define 96 well PCR plate layout on program.



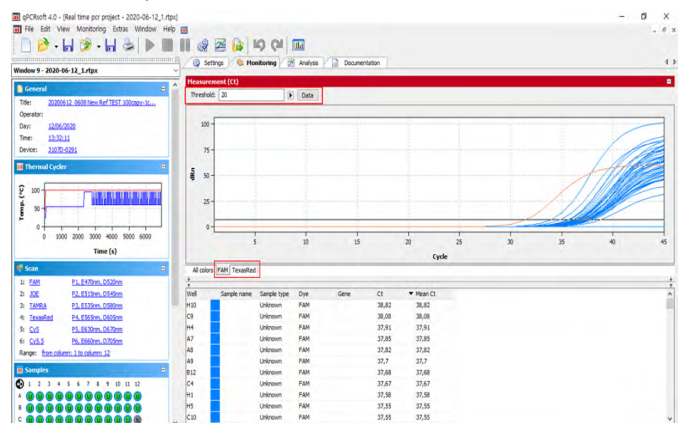
⑤ Click the menu – “Monitoring” and Click “Start Run” button.(▶)



⑥ After finish running, click the menu “Monitoring” and click “Calculate Ct”.



⑦ Check the Threshold, FAM : 20, JOE : 20, and TexasRed : 20. Ct value changes automatically.



All Channel	Threshold						Baseline	
	CFX96	7500	Quantstudio5	qTOWER3	Rotor Gene-Q	LC 480	Begin	End
FAM Texas Red VIC	500	50,000	15,000	20	0.05	Abs Quant/2nd Derivative Max	3	15

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