GeneFinder™

HLA-ABCDRB1DQ Real*Amp* Kit

Instructions for Use

REF IFMR-20.02B006-ABF (Test for Applied Biosystems 7500 Fast)
REF IFMR-20.02B006-ABI (Test for Applied Biosystems 7500)
REF IFMR-20.02B006-QS5 (Test for QuantStudio 5)
REF IFMR-20.02B006-QS6 (Test for QuantStudio 6)
REF IFMR-20.02B006-CFX (Test for CFX96)

IVD

CE 0123

Manufacturer:
OSANG Healthcare Co., Ltd.
Address : 132, Anyangcheondong-ro, Dongan-gu,
Anyang-si, Gyeonggi-do, 14040 Republic of Korea
TEL : +82-31-460-0472
Fax : +82-31-460-9933
E-mail : ohc_cs@osanghc.com
Web site : http://www.osanghc.com/en

EC REP **Obelis S.A**
Bd. General Wahis 53,1030 Brussels, Belgium
Tel:+32-2-732-59-54
Fax:+32-2-732-60-03
E-mail: mail@obelis.net
Web site: https://www.obelis.net

Contents

1. INTRODUCTION

The human leukocyte antigen (HLA) plays a key role in autoimmune disease etiology[1]. As HLA compatibility between donor and recipient is a major factor for determining the outcome of organ and bone marrow transplantations, accurate determination of HLA genotypes is necessary prior to transplantation.

The HLA genes, located on the short arm of human chromosome 6, are well known highly polymorphic. The HLA genes are made up by three different loci, for class-I named A, B and C and while class-II loci are called DP, DQ and DR. The proteins of class-I present on the surface of all cells in the body, and interact with T-cells for reaction or key role for disruption of infected cells, whereas the proteins of class-II lead to cell-mediated immunity under T-cell recognition by interactions with T-cells.

Traditionally, HLA typing has been performed by serological methods[2]. However, this test has been replaced by polymerase chain reaction (PCR) based DNA typing techniques due to its error rate and lack of allele level resolution. As the DNA sequences of the most important HLA alleles are now known, variations in sequences can be identified at the DNA level with the help of synthetic oligonucleotides.[3] However, all molecular genotyping approaches require post-PCR handling which not only increase the required time but, more critically, introduces the potential for cross-contamination of samples and reagents. For this reason, we developed an approach using real-time PCR in conjunction with sequence-specific primers and probes for rapid HLA typing[4]. The real-time PCR is a system to supplement existing faults of methods, so provide information of confirmed inspection process with highly accuracy and sensitivity. It has improved user convenience with rapidity and objective outcomes as well.

2. INTENDED PURPOSE

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit is an in vitro diagnostic medical device and DNA based genotyping method for low-resolution typing of the HLA Loci A, B, C, DRB1 and DQ(DQB1, DQA1) with DNA extracted from human whole blood by using Real-time PCR system.

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit is not automated product, specifically designed for use by qualified healthcare professionals who have received special training in real-time PCR and in vitro diagnostic procedures.

3. TEST PRINCIPLE

Real-time PCR is similar to the conventional PCR, but it is different from that as 'Real-time' quantitative which is monitored the amplification reaction of DNA by camera or detector. Generally there are various technologies to be monitored amplified DNA that probe labeled with fluorescent dye in processing. The genetic copying is increased during PCR reaction, and consequently emission intensity of the fluorescent dye is also specifically increased. Hereby reaction rate and efficiency is facilitated by the fluorescent dye.

Allele specific target DNA is amplified by a forward, reverser primer and *Taq* polymerase. During real-time PCR, DNA-based probe labeled with each fluorescent dye, those are 'fluorescent reporter' and 'fluorescence quencher' at each end of prove can be detected. When both dyes are attached to the probe, emission intensity of reporter is quenched by the fluorescence resonance energy transfer (FRET) of quencher, thus there is no fluorescent signal. As the PCR starts, "primer and probe" bind to the target sequence specifically, and reporter dye is removed by exonuclease activity of *Taq* DNA polymerase then the fluorescent signal begins to appear. Emission intensity of fluorescent dye is

detected by laser. Monitoring of the fluorescence intensities during the PCR run allows the detection and quantitation of the accumulating product in real time. Each step of reaction is implemented by combination of specific HLA target genes conserved regions of internal control. The probe binding with FAM and JOE (VIC) is for detecting each of HLA-ABCDRB1DQ allele, but internal control is bound with ROX (Texas Red) for the detection of fluorescence intensities. The amplification of internal control will be weak or absent by competitive PCR reaction.

4. REQUIRED MATERIALS

1) Materials Provided, Kit Contents

Table 1. Contents of provided materials of the Kit

(Packaging unit: **6 Tests/Kit**)

Label	Cap color	Description	Storage	Quantity
HLA-ABCDRB1DQ *Rxn*	Brown	Reaction mixture	≤-20°C	6 x 984µℓ
HLA-ABCDRB1DQ pol.	Blue	Enzyme	≤-20°C	2 x 60µℓ
HLA-ABCDRB1DQ 96 Well plate	-	Reaction plate	≤-20°C	6 plates
Well plate adhesive film	-	Plate film	–	6 films
Quick Manual (Summary instructions for use)				

Description of the reagents

(1) HLA-ABCDRB1DQ *Rxn*

The Ready-to-Use HLA-ABCDRB1DQ *Rxn* is contained in 1.5ml Amber tube and contain PCR buffer, $MgCl_2$, dNTPs, internal control probe and water.

(2) HLA-ABCDRB1DQ pol.

The Ready-to-Use HLA- ABCDRB1DQ pol. is contained in 0.5mL blue cap tube.

(3) HLA-ABCDRB1DQ 96 Well plate

Each plate is designed for 1 test, consisting of wells that contain dried primer and probe mixtures. Plate layout for each locus is as shown in **Figure 1** and each number is as shown in **Figure 2**

REF IFMR-20.02B006-ABF (0.1ml Optical Plate, test for ABI 7500 Fast)

REF IFMR-20.02B006-ABI (0.2ml White Plate, test for ABI 7500)

REF IFMR-20.02B006-QS5 (0.2ml White Plate, test for QuantStudio 5)

REF IFMR-20.02B006-QS6 (0.2ml White Plate, test for QuantStudio 6)

REF IFMR-20.02B006-CFX (0.1ml White Plate, test for CFX96)

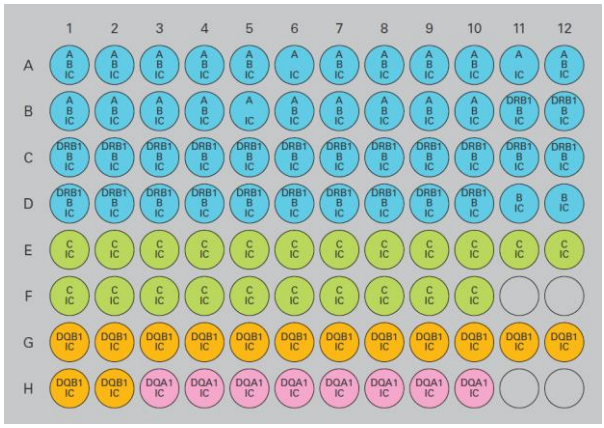


Figure 1.

HLA-ABCDRB1DQ 96 well plate target position

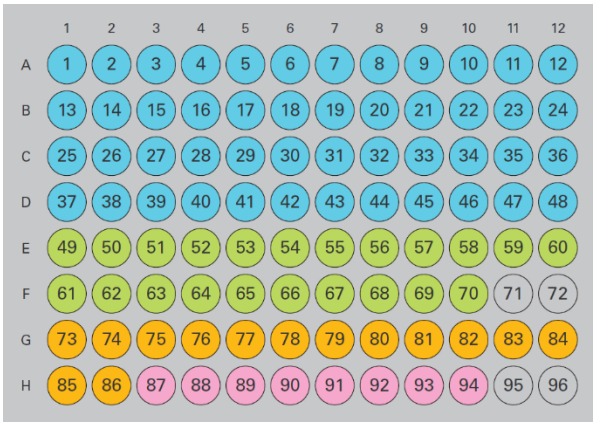


Figure 2.

HLA-ABCDRB1DQ 96 well plate position number

(4) Well plate adhesive film

Plate adhesive film is used to prevent evaporation of product during the PCR process.

2) Materials Required, but Not Provided

- Applied Biosystems® 7500 Real Time PCR Instrument System
- Applied Biosystems® 7500 Fast Real Time PCR Instrument System
- Quantstudio™ 5 Real-Time PCR System
- Quantstudio™ 6 Flex Real-Time PCR System
- Bio-Rad CFX96 Real-Time PCR detection system
- DNA isolation Kit: Use of QIAamp DNA blood mini Kit (QIAGEN, Germany, Cat. # 51104) is recommended.
- Pipettes (1- 20㎕, 20-200㎕, 200-1,000㎕)

Note. Recommend electronic single-channel dispenser capable of dispensing 10㎕ aliquots for adding the Master Mixture to the wells.

- Disposable pipette tips
- Vortex mixer
- Microcentrifuge
- Powder-free gloves (disposable)
- Table top centrifuge for 96 well PCR plates
- Real-Time PCR Instrument System

5. STORAGE AND HANDLING REQUIREMENT

- The kit must be stored below -20°C .
- Expires 12 months after manufacturing. Do not use past the expiration date.
- Expires 6 weeks after opening the pouch. Do not use after In-use life time. Any unused PCR plates should be kept in their original pouch, resealed with tape to prevent moisture accumulation during storage.
- Store the rest of the kit below -20°C. Unnecessary repeated freezing and thawing will be occurred inaccurate results.
- The kit is stable until the expiry date on the label.

6. WARNINGS AND PRECAUTIONS

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit is designed for **In vitro diagnostics.**

General warnings

- This product should be **used by professionals** in the laboratory.
- All reagents should be handled in accordance with good laboratory practice using appropriate precautions. In addition, handle all patient samples as potentially infectious.
- Do not use reagents past the expiration date printed on the label.
- Do not use reagents with any evidence of turbidity or microbial contamination.
- Do not mix reagent from different lot.
- Do not use HLA-ABCDRB1DQ 96 Well plate have defect or damage.

Biohazard Warnings

- All blood products should be treated as potentially infectious. No known test method(s) can offer assurance that products derived from human blood will not transmit infectious agents.
- All used PCR blocks should be treated as potentially infectious and should be destroyed according to the valid national guidelines.
- Decontaminate and dispose of all specimens, reagents and other potentially contaminated materials in accordance with local regulations.
- Use universal precautions when performing the assay. Handle samples as if capable of transmitting infection.
- Wear personal protective apparel, including disposable gloves, throughout the assay procedure. Thoroughly wash hands after removing gloves, and dispose of gloves as biohazardous wastes.
- Waste all materials contact with biological samples by high temperature sterilization (120°C, 1 hour)
- Use aerosol-resistant pipette tips and use a new tip every time a volume is dispensed.
- Store the Kit away from any source of contaminating DNA, especially amplified nucleic acid.
- Use sterile disposable laboratory materials and do not reuse.
- Do not modify reagents or volume of reagents randomly and use other method not recommended.
- See Material Safety Data Sheet for detailed information

7. PROCEDURE

7.1 Blood Sample Collection

The Whole blood can be collected using blood collection tubes with sodium citrate or EDTA as anticoagulation. Heparin containing blood can interfere with the PCR amplification reaction. For this reason, heparinized blood should not be used as a starting material for DNA extraction. Please refer to manufacturer's instruction for blood sample storage and stability.

Ref. Effects of heparin on polymerase chain reaction for blood white cells. J Clin Lab Anal. 1999; 13(3): 133-40

7.2 DNA Isolation

It is recommended using commercial DNA extraction Kit such as Qiagen DNA blood mini Kit (Qiagen, Germany, Cat # 51104).

7.3 DNA Quantity

The DNA sample should be re-suspended in sterile distilled water or TE buffer solution at a concentration of 50-100 ng/㎕.

7.4 DNA Purity

The DNA sample should have an A260/A280 ratio between 1.6 and 2.0. DNA samples may be used immediately after isolation. There is no adverse effects on the HLA typing results whether it is stored at -20°C or below for extended period of time (within 1 year).

The purity and concentration of DNA is of decisive importance for optimal test results.

7.5 Sample Preparation

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit is used with genomic DNA samples extracted from blood with EDTA. Do not use blood sample in heparin, which can lead to PCR reaction inhibition. The blood samples should be collected according to guidelines. Blood samples can be stored at 2-8°C for 1 week but use of fresh blood samples when extracting DNA is recommended for precise testing.

7.6 Reagent Preparation and PCR Amplification

1) Remove the tube of pre-aliquoted HLA-ABCDRB1DQ *Rxn* from the Kit and thaw at room temperature (20°C -25°C). Each tube is for one sample test.
2) Once the HLA-ABCDRB1DQ *Rxn* is thawed, vortex to mix well and centrifuge briefly to collect all liquid to the bottom.
3) Take a HLA-ABCDRB1DQ 96 Well plate from the pouch in the Kit. Any unused plate must be stored in the pouch and sealed for future use. Each plate is for one sample test.
4) Preparation of Master Mixture for PCR amplification by mixing as below (Table 2).

Table 2. Preparation of Mater Mixture

(Unit: ㎕)

Components	volume of reagents
HLA-ABCDRB1DQ *Rxn*	984
HLA-ABCDRB1DQ pol.	16
DNA (50~100 ng/㎕)	100
Total	1100

① Remove HLA-ABCDRB1DQ pol. from the Kit and keep on ice immediately.

② Add 16㎕ of HLA-ABCDRB1DQ pol. to the HLA-ABCDRB1DQ *Rxn* tube.

③ Add 100㎕ of DNA sample (50-100 ng/㎕) to the HLA-ABCDRB1DQ *Rxn* tube. Vortex to mix well and centrifuge to collect all components to the bottom.

5) Dispense 10㎕ of the master mix into wells of the HLA-ABCDRB1DQ 96 Well plate **(1 Test/1 Plate)**. Usage of an electronic single-channel dispenser is recommended.

Caution. Care should be taken to avoid cross-contamination between wells and not to contact the dried primers and probes pellet at the bottom of each well with pipette tip.

6) Seal the HLA-ABCDRB1DQ 96 Well plate with adhesive film and check that all reaction wells are completely covered to prevent evaporative loss during the PCR process. Centrifuge for 2 minutes at 2000 rpm and make sure the dispensed solution comes in contact with the dried primers & probes on bottom of each well of plate.

7) Place the PCR plate on Real-Time PCR instrument.

8) Perform PCR amplification.

7.7 Instrument

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit is optimized for the following equipment.

Table 3. Applicable equipment

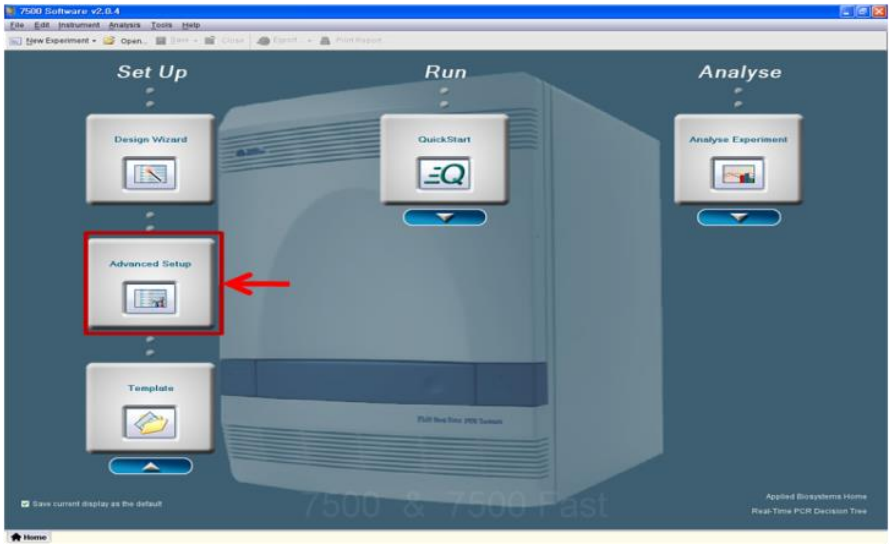
Trade name (Model no.)	Catalogue no.	Compatible Instrument
GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit (IFMR-20)	IFMR-20.02B006-ABF	ABI7500 Fast
	IFMR-20.02B006-ABI	ABI7500
	IFMR-20.02B006-QS5	QuantStudio™ 5
	IFMR-20.02B006-QS6	QuantStudio™ 6
	IFMR-20.02B006-CFX	CFX96™

7.8.1 Condition setting and implementation (ABI 7500 fast / ABI 7500)

Operate PCR instrument according to the manufacturer's manual prior to amplification.

I. Template input

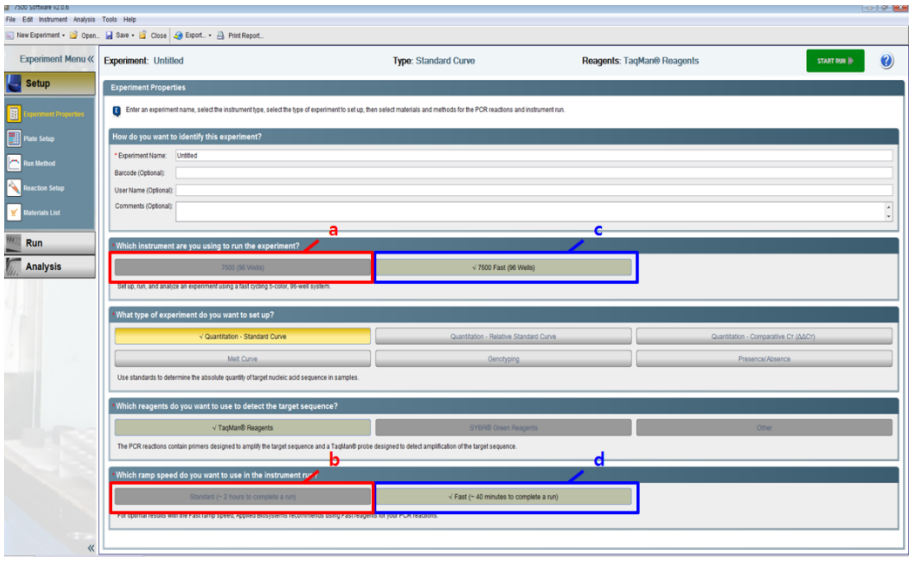
1> Select 'Advanced Setup' as below figure



2> Refer to figure below, Set the Experiment Properties according to '**Table 3.**' below

Table 3. Experiment Properties

Instrument	Experiment	Ramp speed
ABI7500	**a.** 7500 (96Wells)	**b.** Standard
ABI7500 Fast	**c.** 7500 Fast (96Wells)	**d.** Fast



3> Refer to figure below, take things in order

1. Select 'Plate Setup' mode
2. Choose the 7 targets by 'Add New Target', input target names (refer to '**Table 4.**' below)
3. Select dye for each target according to information of Reporter and Quencher
4. Choose the Sample by 'Add New Sample', input sample name

5. Move to 'Assign Targets and Samples'

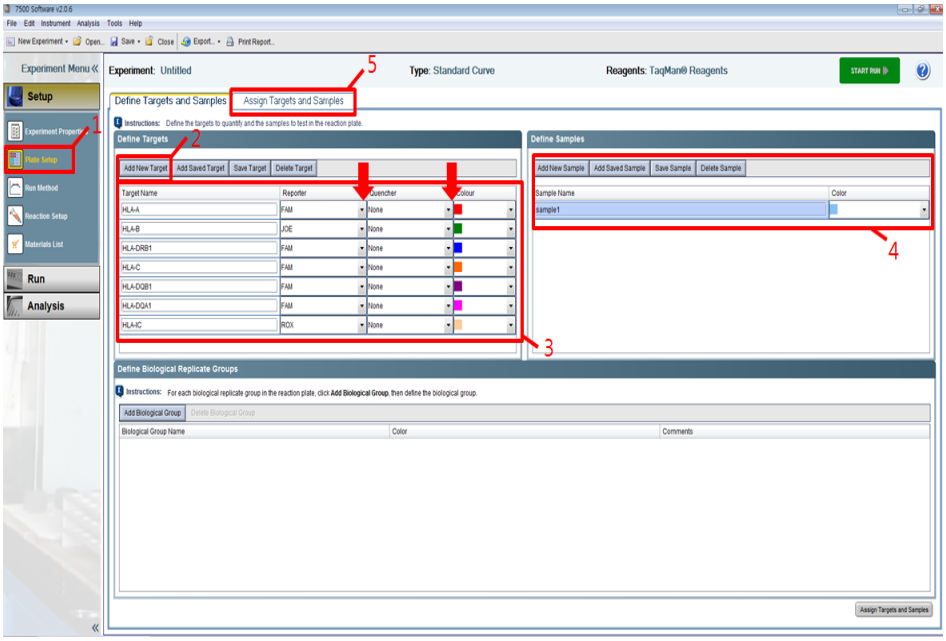
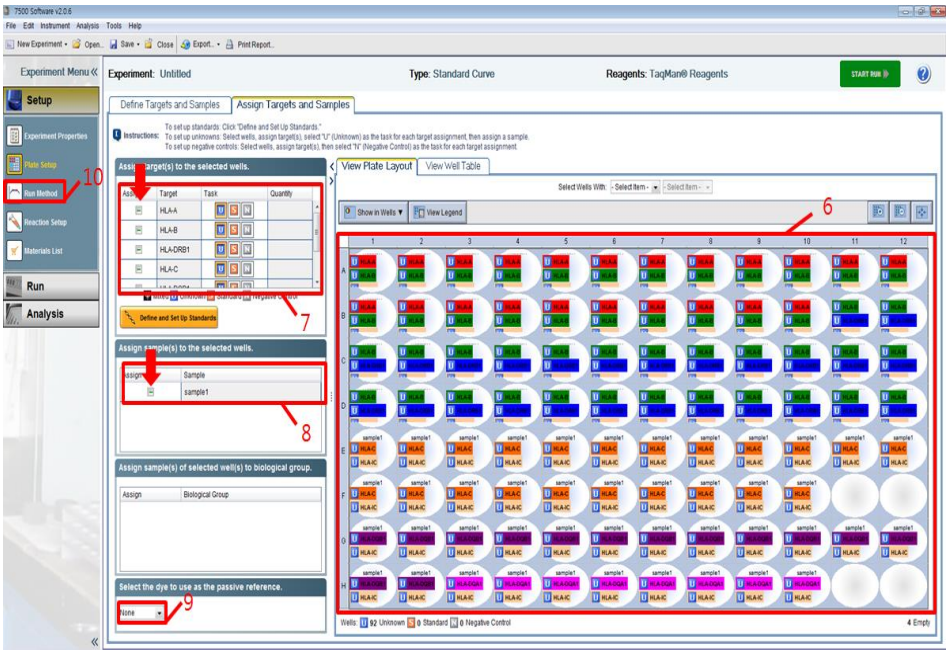


Table 4. Fluorescence setting

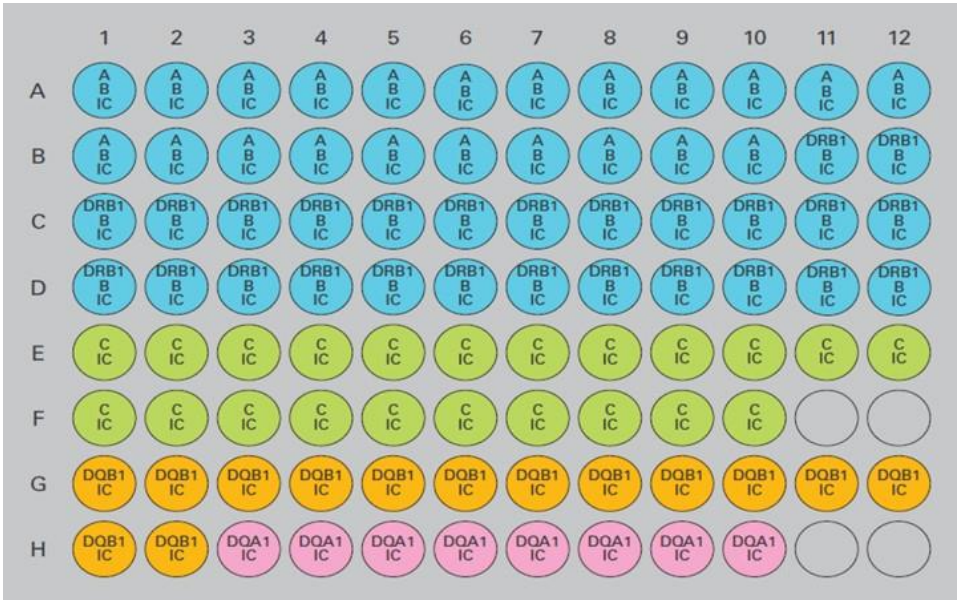
Target	Reporter
A	FAM
B	JOE
C	FAM
DRB1	FAM
DQB1	FAM
DQA1	FAM
IC	ROX

4> Refer to figure below, take things in order

6. Choose the target position of the 'View Plate Layout'
7. Mark box the all targets of detector
8. Choose the plate well position of sample, then select box according to samples.
9. Choose 'None' on screen of selection for Passive reference dye
10. Move to 'Run Method'



Note: The software settings should adhere to the image below, paying attention to A6, A11, B5, D11, and D12.



5> Refer to figure below, take things in order

11. Input the PCR condition according to '**Table 5.** Real-time PCR condition'

11-a. Click the data collection then 'on' mode

11-b. Input Total volume

11-c. Check the number of cycles

12. Save the PCR condition as 'Save As Template'

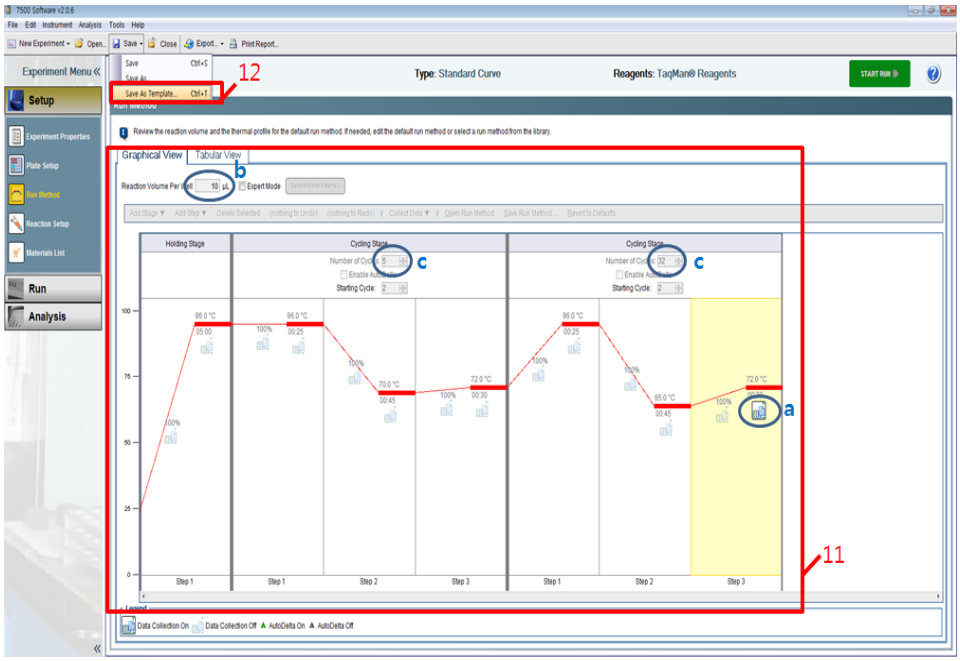


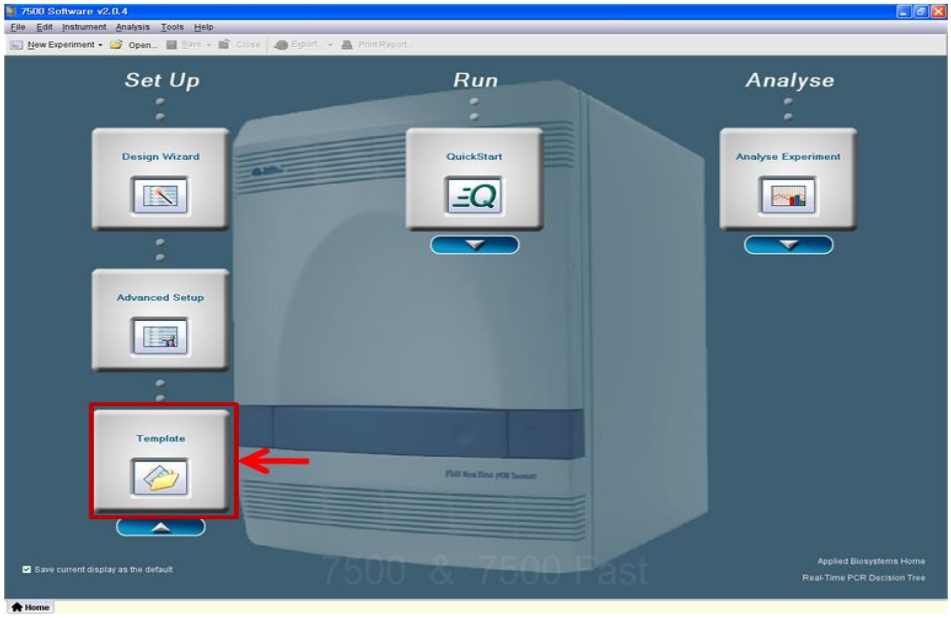
Table 5. Real-Time PCR condition

Cycle	Stage	Temp.	Time
1 cycle	Pre-denaturation	96 ºC	5 min
5 cycles	Denaturation	96 ºC	25 sec
	Annealing	70 ºC	45 sec
	Extension	72 ºC	30 sec
32 cycles	Denaturation	96 ºC	25 sec
	Annealing	65 ºC	45 sec
	Extension*	72 ºC	30 sec

***Note**. *72 °C at 32 cycles-Extension step have to be Data collection.*

II. Saved Template Importing

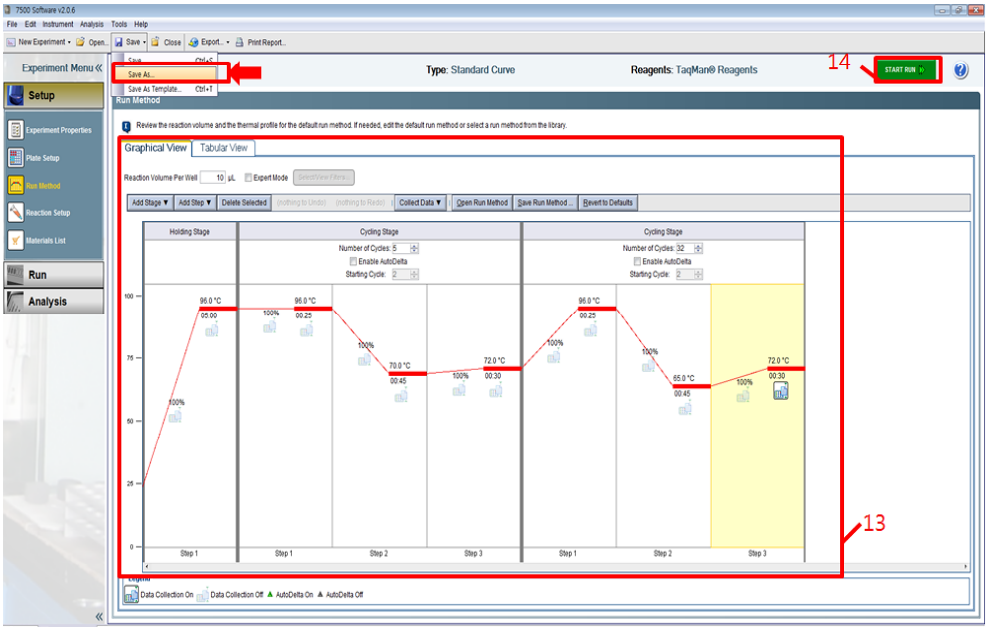
1> Select 'Template' as figure below



2> Start program with saved template

3> Refer to figure below, take things in order

13. Confirm again saved PCR condition
14. Save the data with 'START RUN' in the upper right of the screen, if the 'START RUN' button is not active, save the data by clicking 'Save as' of the menu then click the 'START RUN' again



III. Analysis Setting

1. Select 'Analysis Settings' as below figure
2. In analysis setting, check the 'Edit Default Setting' (Blue box-a) and clear the 'Automatic Threshold' and 'Automatic Baseline' (Blue box-b) after that input the threshold & baseline according to '**Table 6**'

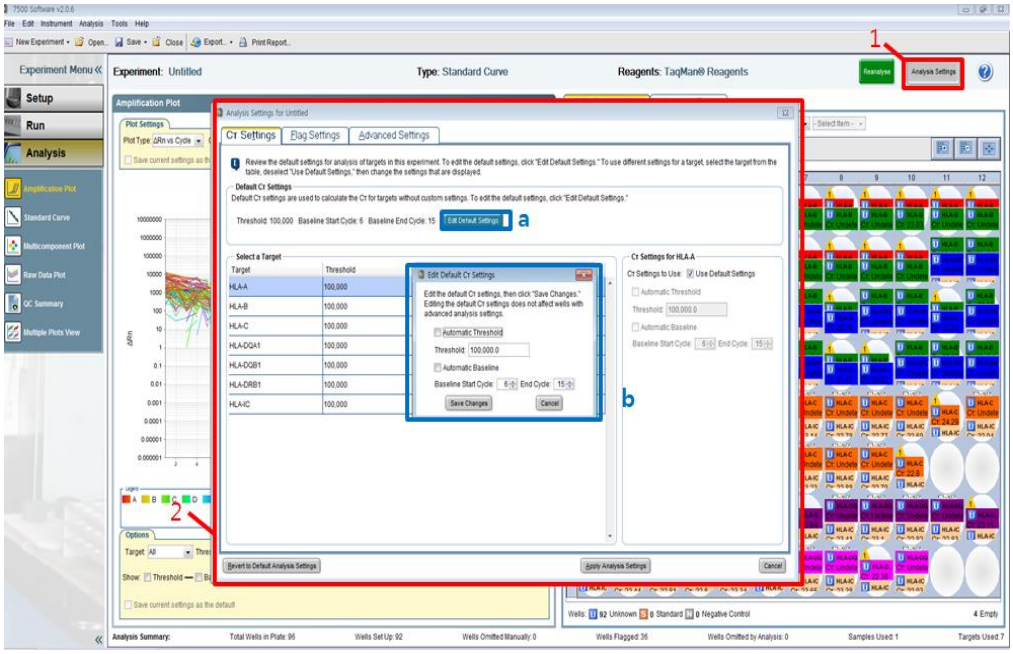


Table 6. Analysis setting

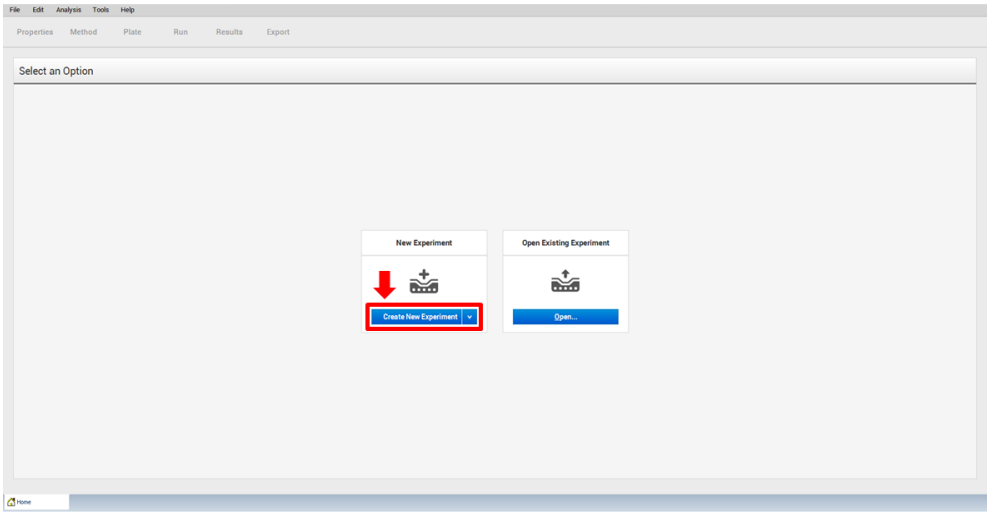
Real time PCR	Target	Threshold	Baseline	
			Start	End
ABI7500 Fast	All	80,000	6	15
ABI7500	All	150,000	6	15

7.8.2 Condition setting and implementation (QuantStudio™ 5)

Operate PCR instrument according to the manufacturer's manual prior to amplification.

I. Template input

1> Select 'Create New Experiment' as figure below



2> Refer to figure below, Set the Experiment Properties.

1. Choose the items according to '**Table 7.** Experiment Properties'

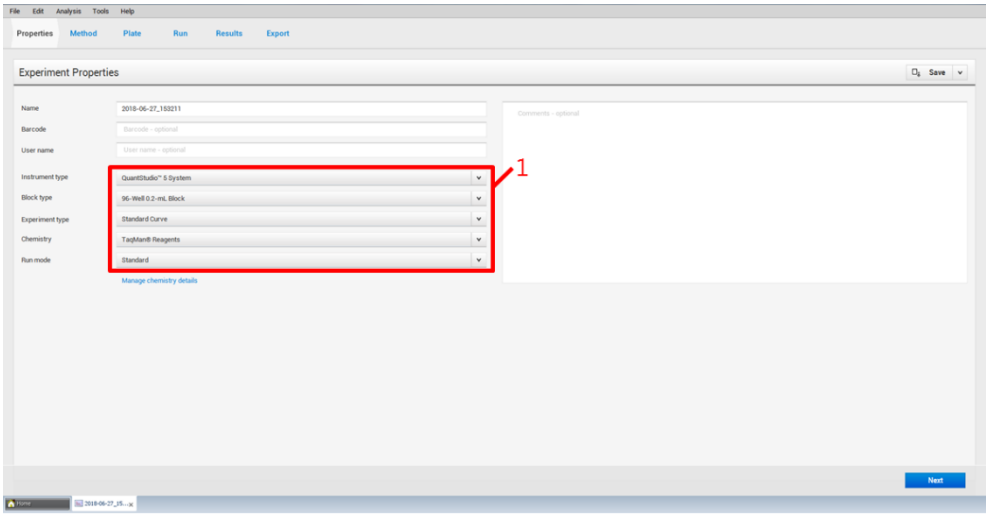


Table 7. Experiment Properties

Instrument type	QuantStudio™ 5 System
Block type	96-Well 0.2-mL Block
Experiment type	Standard Curve
Chemistry	TaqMan® Reagents
Run mode	Standard

3> Refer to figure below, take things in order

2. Input the PCR condition according to '**Table 8.** Real-time PCR condition'

2-a. Click the data collection then 'on' mode

2-b. Input Total volume

2-c. Check the number of cycles

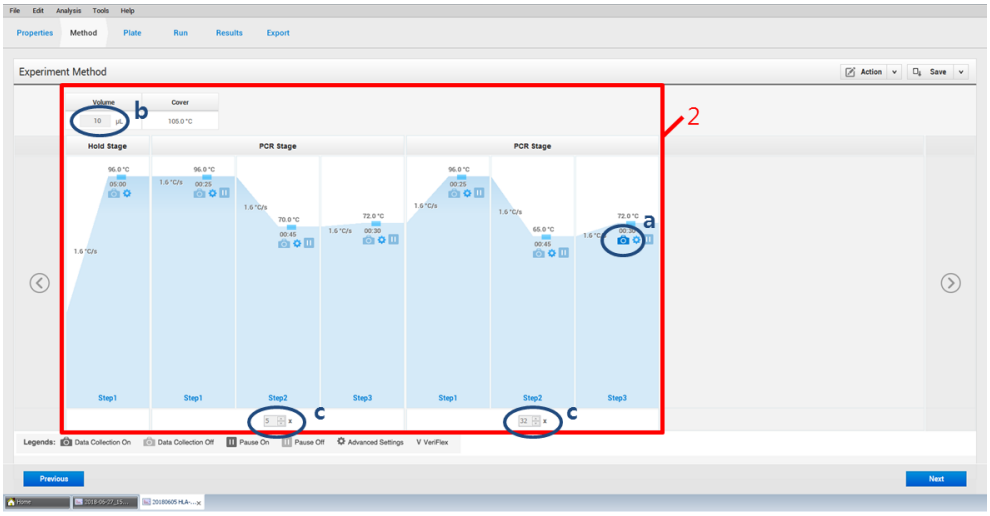


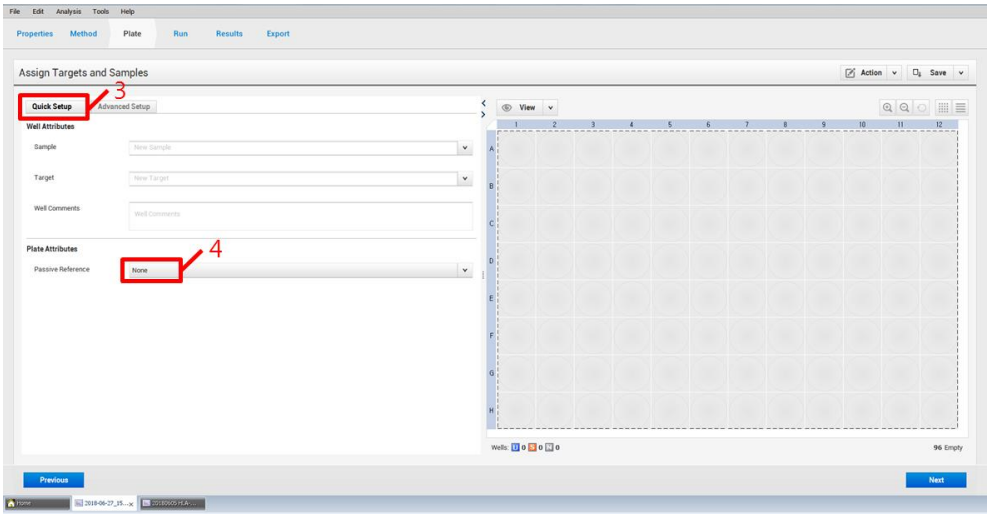
Table 8. Real-Time PCR condition

Cycle	Stage	Temp.	Time
1 cycle	Pre-denaturation	96 ºC	5 min
5 cycles	Denaturation	96 ºC	25 sec
	Annealing	70 ºC	45 sec
	Extension	72 ºC	30 sec
32 cycles	Denaturation	96 ºC	25 sec
	Annealing	65 ºC	45 sec
	Extension*	72 ºC	30 sec

Note. *72 °C at *32 cycles-Extension step have to be Data collection.*

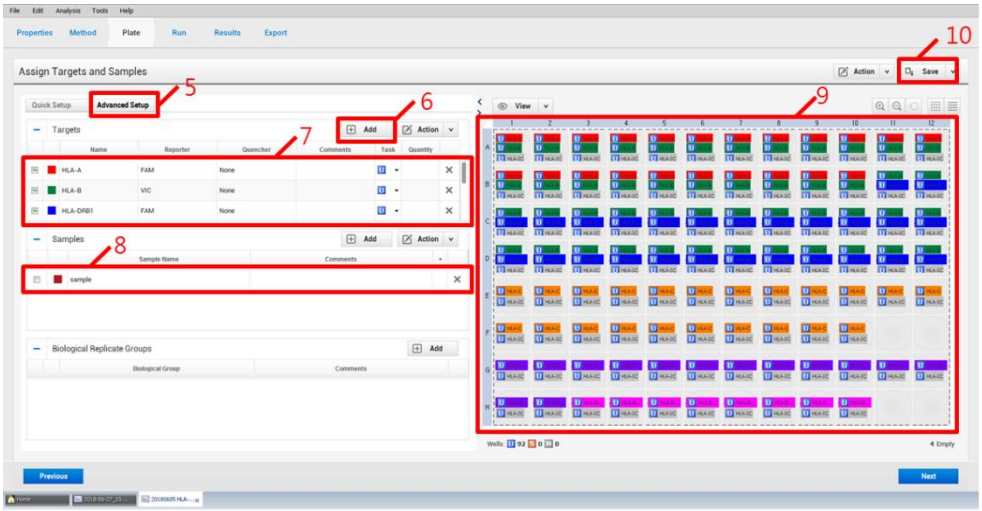
4> Refer to figure below, take things in order

3. Select 'Quick Setup' mode.
4. Choose 'None' on screen of selection for Passive reference dye



5> Refer to figure below, take things in order

5. Select 'Advanced Setup' mode.
6. Press 'Add' to create a total of 7 target items.
7. Input target names and select dye for each target according to information of Reporter and Quencher (refer to '**Table 9.** Targets' below)
8. Input sample name
9. Choose the target position of the 'Plate Layout'. Set F11, F12, H11, H12 well to clear.
10. Save the PCR condition as 'Save' and 'Save as...'



Note: The software settings should adhere to the image below, paying attention to A6, A11, B5, D11, and D12.

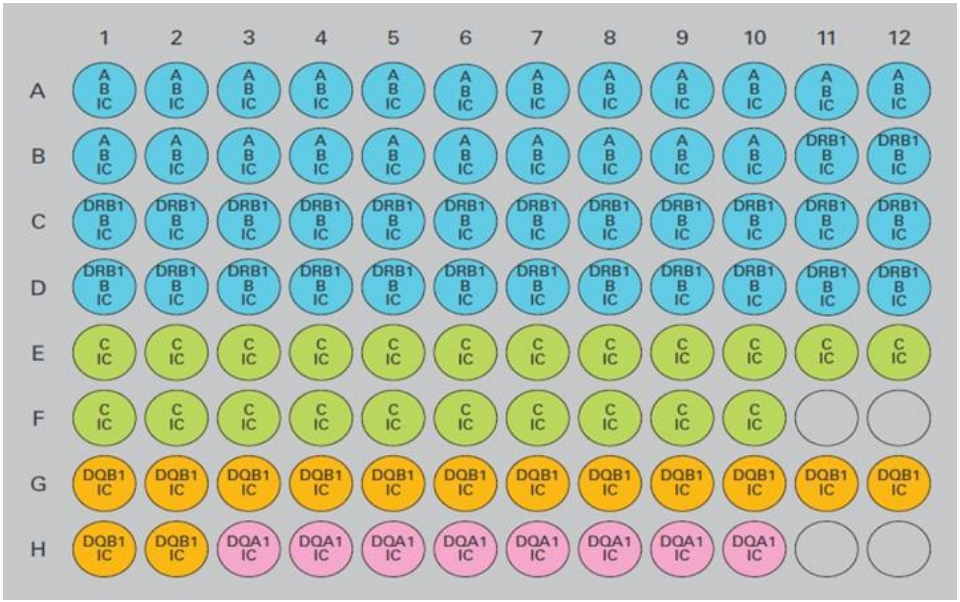
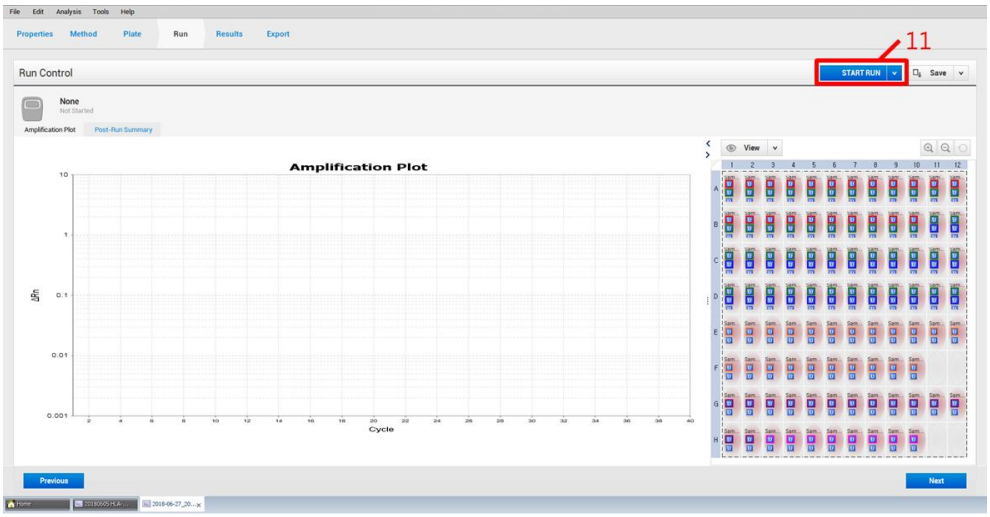


Table 9. Targets

Name	Reporter	Quencher	Task
HLA-A	FAM	None	U
HLA-B	VIC	None	U
HLA-C	FAM	None	U
HLA-DRB1	FAM	None	U
HLA-DQB1	FAM	None	U
HLA-DQA1	FAM	None	U
HLA-IC	ROX	None	U

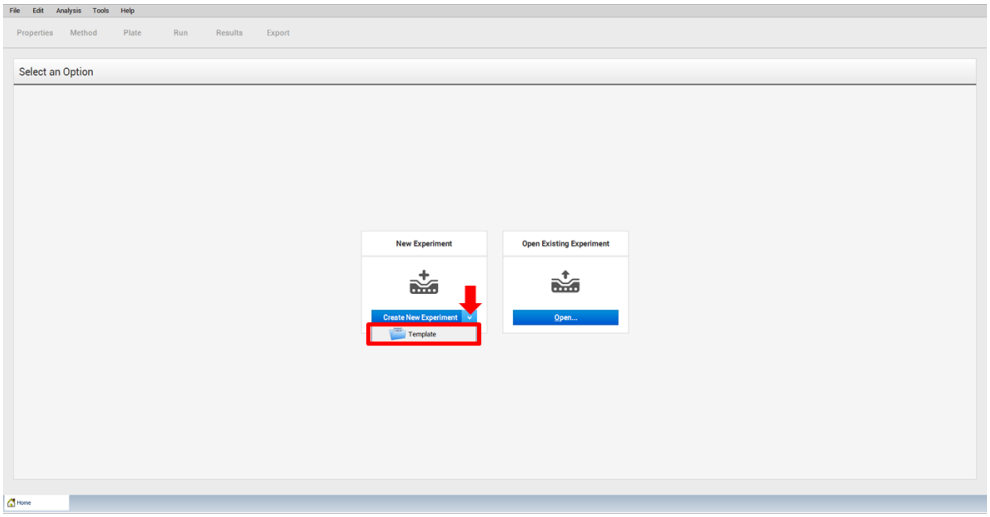
6> Refer to figure below, take things in order

11. Select the device in 'START RUN' and check the location to save the file (*.eds). And make sure PCR running start.



II. Saved Template Importing

1> Select 'Template' as figure below

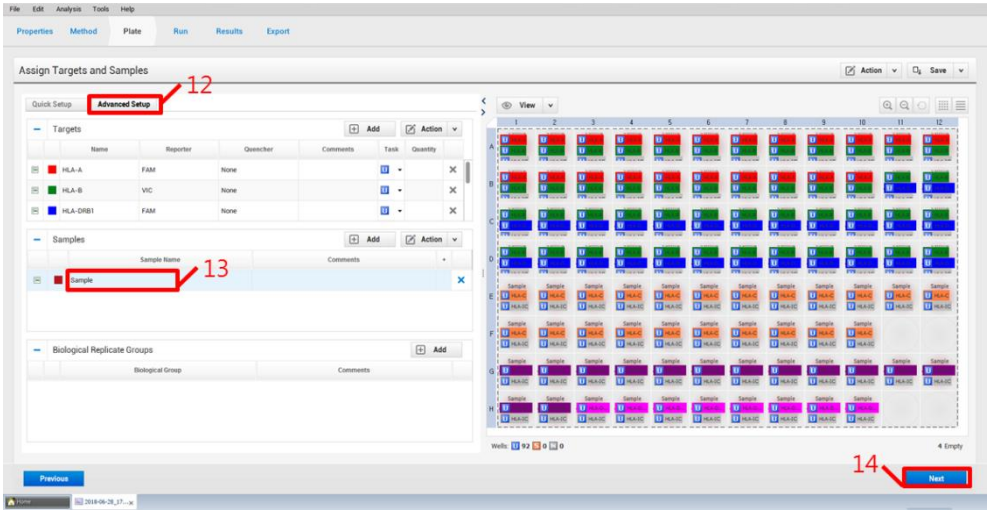


2> Start the program using saved template and confirm again saved 'Properties', 'Method' and click 'Next'.

(according to '**Table 8.** Real-time PCR condition')

3> Refer to figure below, take things in order

12. Move to 'Advanced Setup' as below figure
13. Input sample name
14. Press 'Next' to move to the next step.

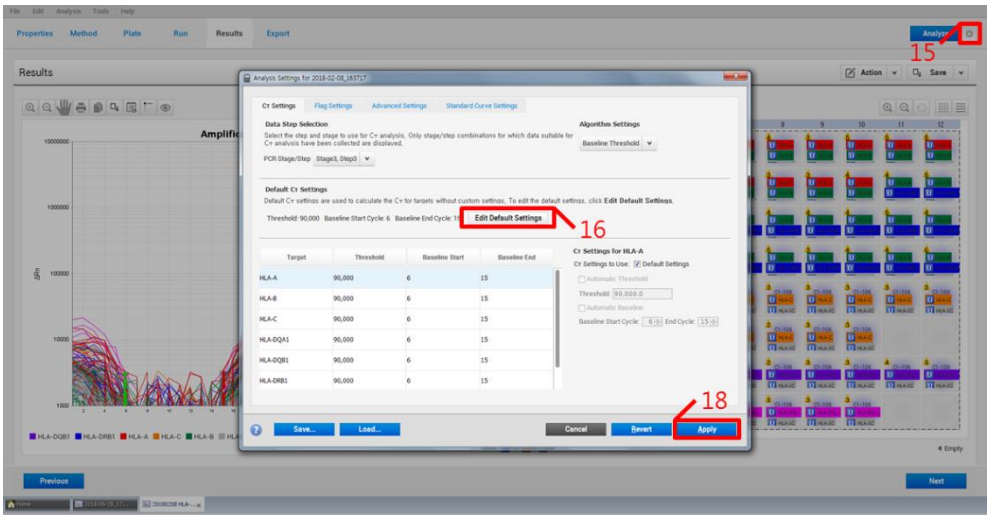


4> Select the device in 'START RUN' and check the location to save the file (*.eds). And make sure PCR running start.

III. Analysis Setting

1> Refer to figure below, take things in order

15. Select 'Analysis Settings' as below figure
16. In analysis setting, check the 'Edit Default Setting'
17. Clear the 'Automatic Threshold' and 'Automatic Baseline' after that input the threshold & baseline according to '**Table 10**' and execute 'Save Changes'.
18. Click 'Apply' to accept the set analysis settings.



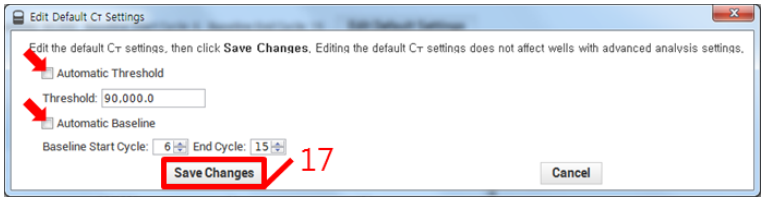


Table 10. Analysis setting

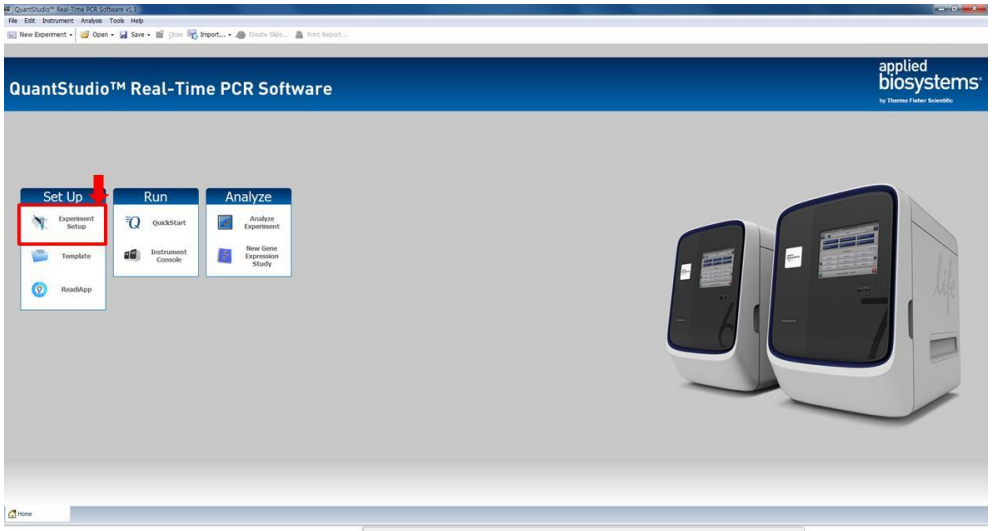
Real time PCR	Target	Threshold	Baseline	
			Start	End
QuantStudio™ 5	All	90,000	6	15

7.8.3 Condition setting and implementation (QuantStudio™ 6)

Operate PCR instrument according to the manufacturer's manual prior to amplification.

I. Template input

1> Select 'Experiment Setup' as below figure



2> Refer to figure below, Set the Experiment Properties.

1. Choose the items according to '**Table 11.** Experiment Properties'.

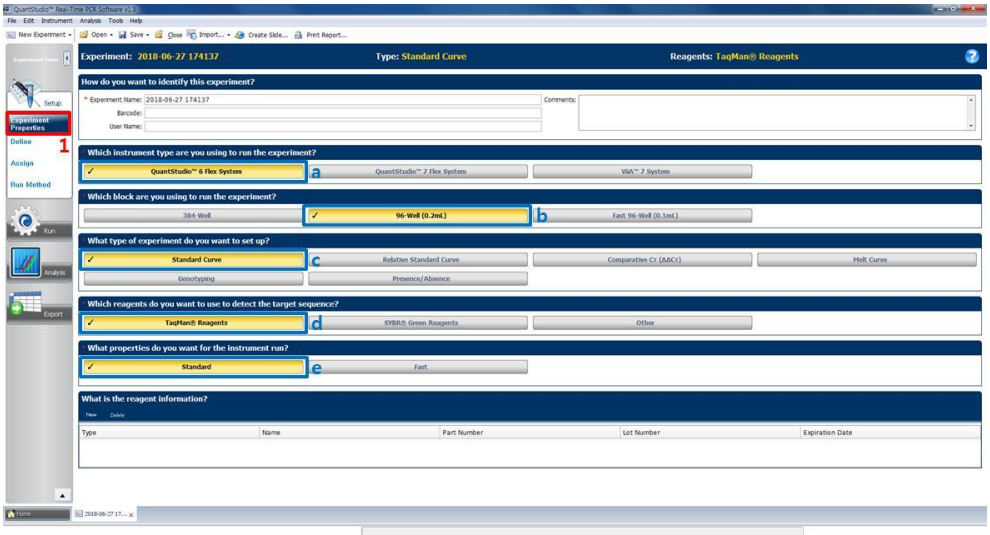


Table 11. Experiment Properties

Instrument type	QuantStudio™ 6 Flex System
Block type	96-Well (0.2mL)
Experiment type	Standard Curve
Chemistry	TaqMan® Reagents
Run mode	Standard

3> Refer to figure below, take things in order.

2. Select 'Define' mode.

3. Click 'Add' to create a total of 7 target items.

4. Input target names and select dye for each target according to information of Reporter and Quencher (refer to '**Table 12.** Targets' below).

5. Input sample name.

6. Choose 'None' on screen of selection for Passive reference dye.

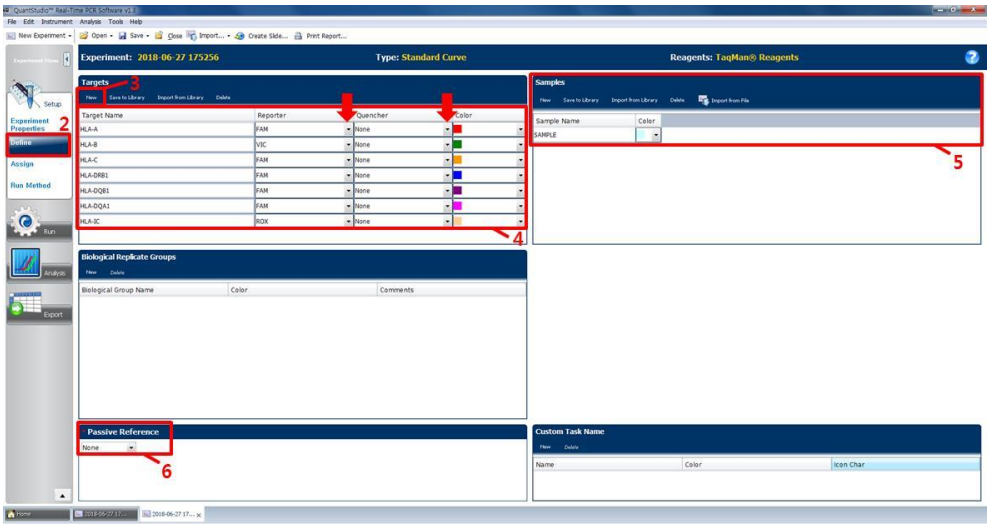


Table 12. Targets

Name	Reporter	Quencher
HLA-A	FAM	None
HLA-B	VIC	None
HLA-C	FAM	None
HLA-DRB1	FAM	None
HLA-DQB1	FAM	None
HLA-DQA1	FAM	None
HLA-IC	ROX	None

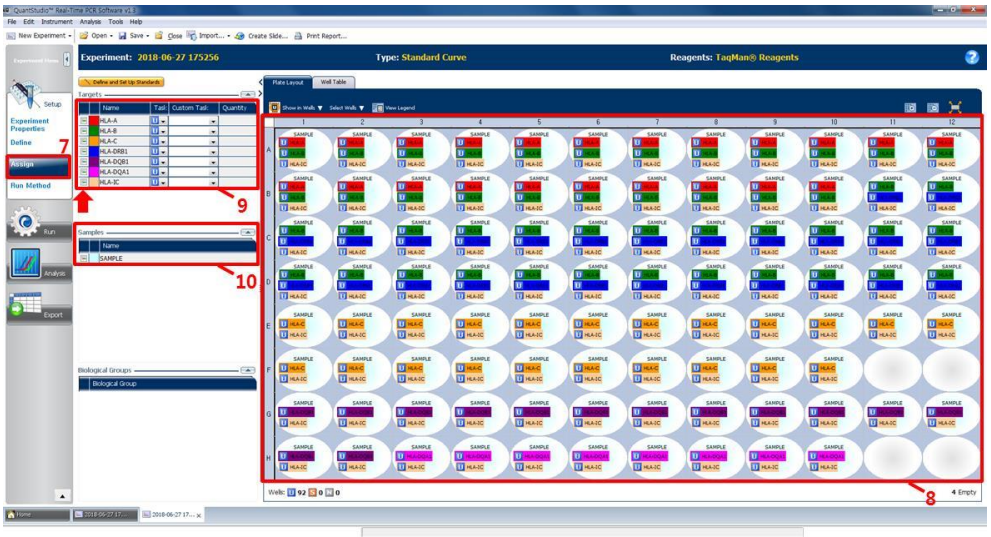
4> Refer to figure below, take things in order.

7. Select 'Assign' mode.

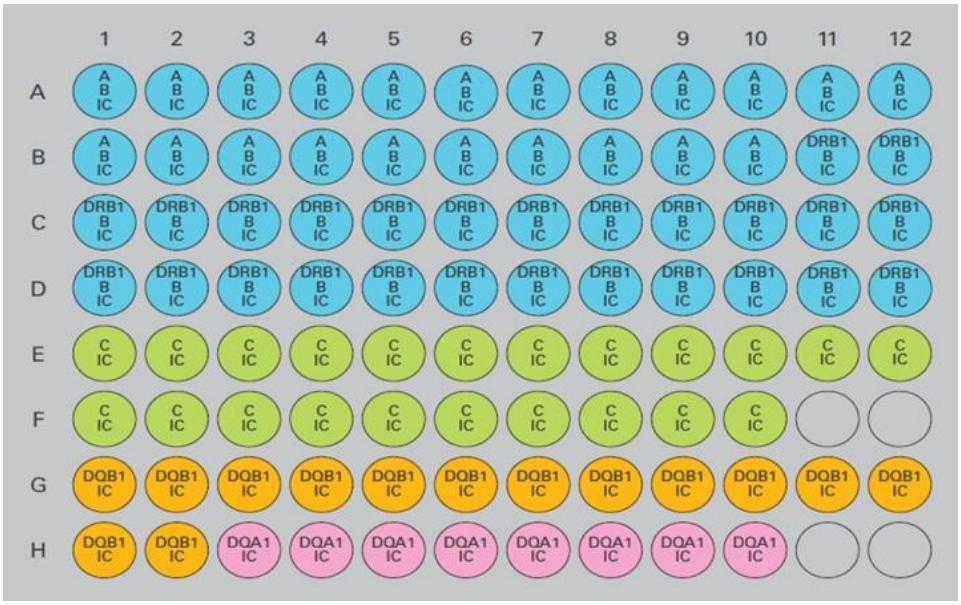
8. Choose the target position of the 'Plate Layout'.

9. Mark box the all targets of detector. Set F11, F12, H11, H12 well to clear.

10. Choose the plate well position of sample, then select box according to samples.



Note: The software settings should adhere to the image below, paying attention to A6, A11, B5, D11, and D12.



5> Refer to figure below, take things in order.

11. Select 'Run method' mode.
12. Input the PCR condition according to '**Table 13.** Real-time PCR condition'.
    12-a. Click the data collection then 'on' mode
    12-b. Input Total volume
    12-c. Check the number of cycles

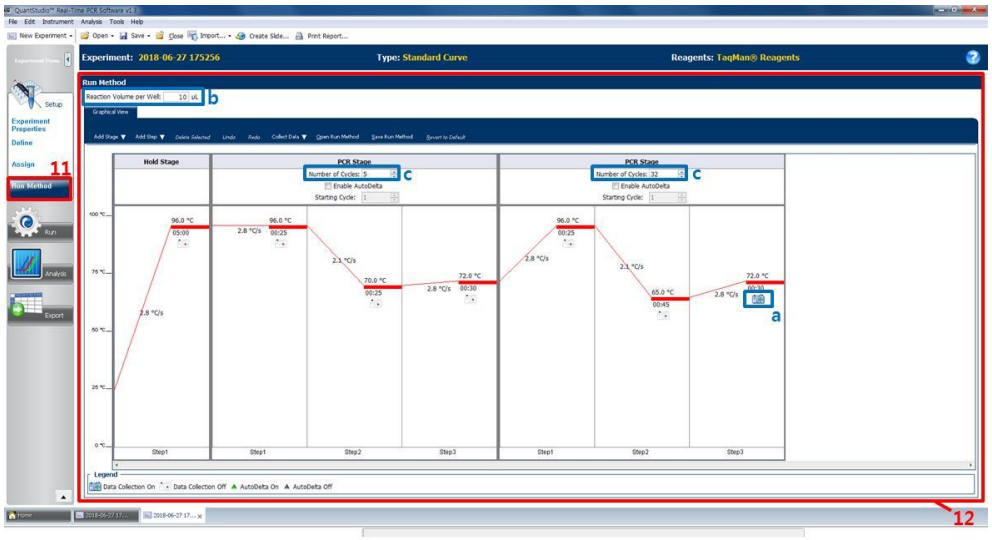


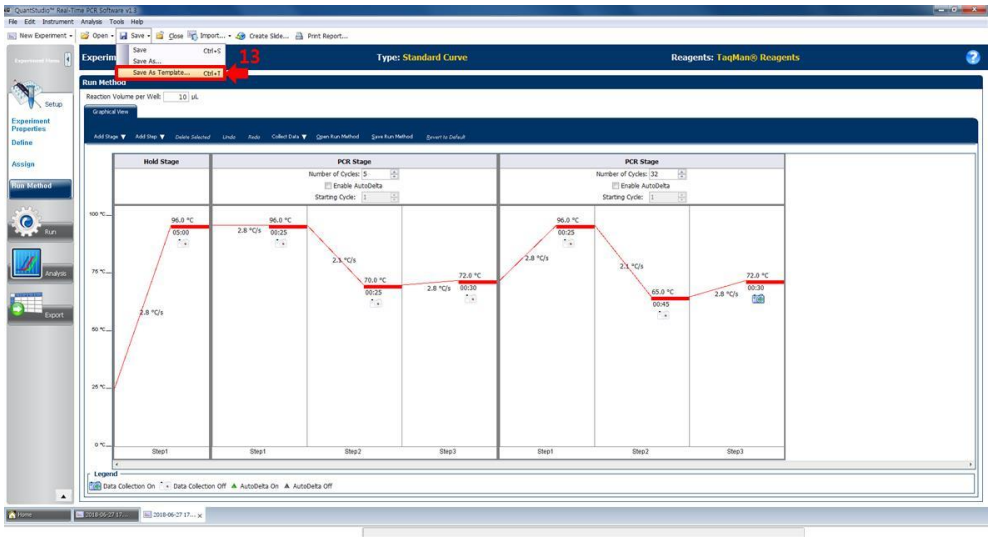
Table 13. Real-Time PCR condition

Cycle	Stage	Temp.	Time
1 cycle	Pre-denaturation	96 ºC	5 min
5 cycles	Denaturation	96 ºC	25 sec
	Annealing	70 ºC	45 sec
	Extension	72 ºC	30 sec
32 cycles	Denaturation	96 ºC	25 sec
	Annealing	65 ºC	45 sec
	Extension*	72 ºC	30 sec

Note. *72 °C at *32 cycles-Extension step have to be Data collection.*

6> Refer to figure below, take things in order

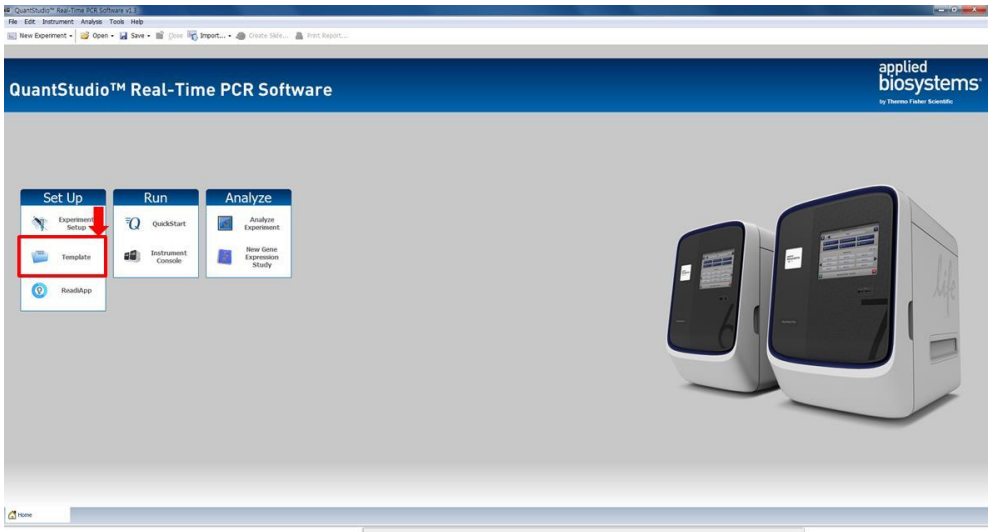
13. Save the PCR condition as 'Save As Template'



7> Select 'Run' mode. Click the device in 'START RUN' and check the location to save the file (*.eds). And make sure PCR running start.

II. Saved Template Importing

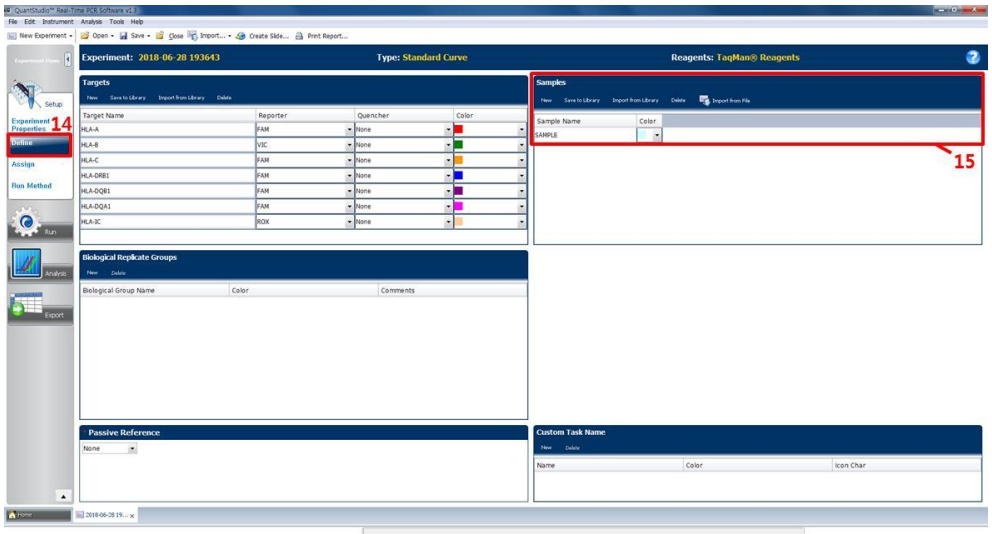
1> Select 'Template' as figure below



2> Start the program using saved template and confirm again saved 'Properties', 'Method' (according to '**Table 13.** Real-time PCR condition).

3> Refer to figure below, take things in order.

14. select 'Assign' mode.
15. Input sample name.



4> Select 'Run' mode. Click the device in 'START RUN' and check the location to save the file (*.eds). And make sure PCR running start.

III. Analysis Setting

16. Select 'Analysis Settings' as below figure.
17. In analysis setting, check the 'Edit Default Setting' (Blue box-a) and clear the 'Automatic Threshold' and 'Automatic Baseline' (Blue box-b) after that input the threshold & baseline according to '**Table 14**'.

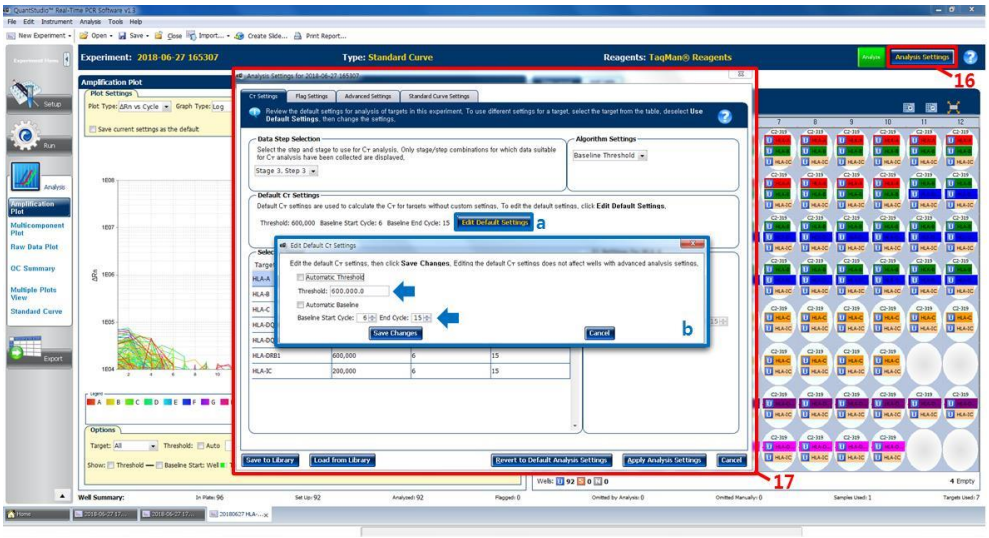


Table 14. Analysis setting

Real time PCR	Target	Threshold	Baseline	
			Start	End
QuantStudio™ 6	A, B, C, DRB1, DQB1, DQA1	600,000	6	15
	IC	200,000		

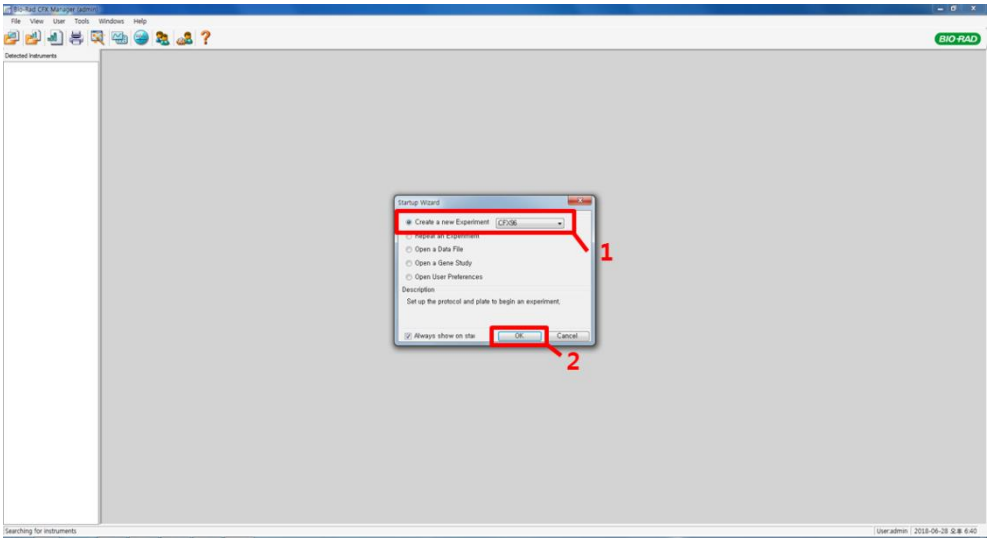
7.8.4 Condition setting and implementation (CFX96™)

Operate PCR instrument according to the manufacturer's manual prior to amplification.

I. Template input

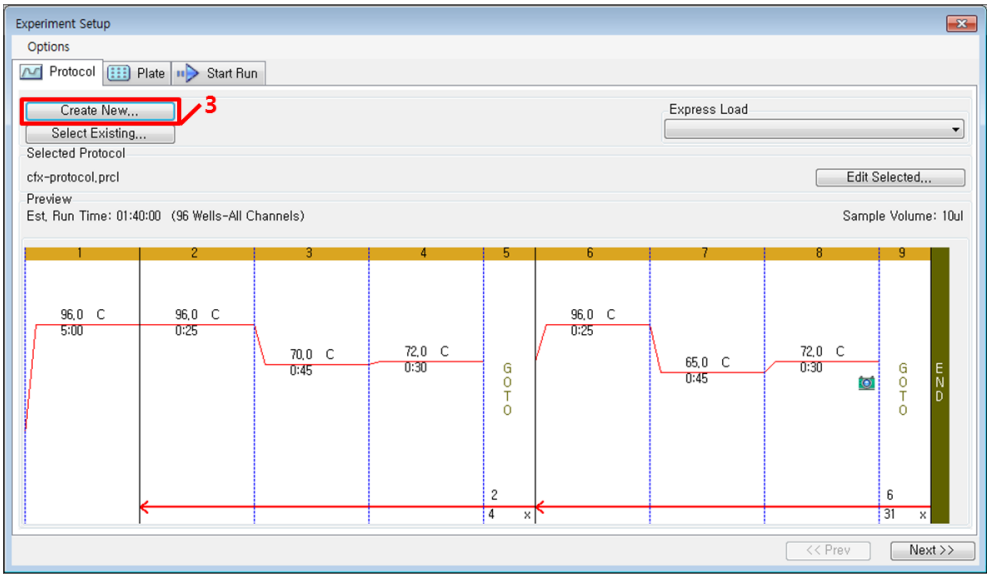
1> Refer to figure below, take things in order

1. Select 'Create a new Experiment CFX96' as below figure.
2. Press 'OK' to proceed to 'Experiment Setup'.



2> Refer to 'Experiment Setup' figure below, take things in order

3. To create a new protocol, enter 'Create New'.



3> Refer to 'Protocol Editor' figure below, take things in order

4. Input Sample Volume 10 ㎕.

5. Input the PCR condition according to '**Table 15.** Real-time PCR condition'

6. Click 'OK' to save the Protocol File (*.prcl).

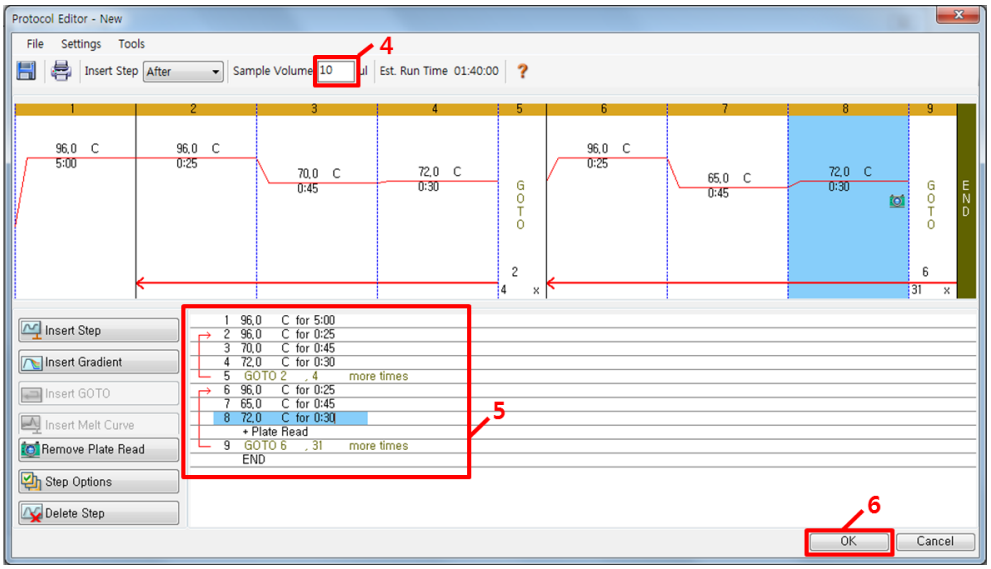


Table 15. Real-Time PCR condition

	Step	Temperature	Time
1	Denaturation	96 ºC	5 min
2	Denaturation	96 ºC	25 sec
3	Annealing	70 ºC	45 sec
4	Extension	72 ºC	30 sec
5	GOTO 2,	4 more times	
6	Denaturation	96 ºC	25 sec
7	Annealing	65 ºC	45 sec
8	Extension*	72 ºC	30 sec
9	GOTO 6,	31 more times	

Note. *Select ON Add Plate Read to Step.

4> Refer to 'Plate Editor' figure below, take things in order

7. In 'Settings', select Plate size ✓ 96 Wells, Plate type ✓ BR white and Units ✓ copy number.

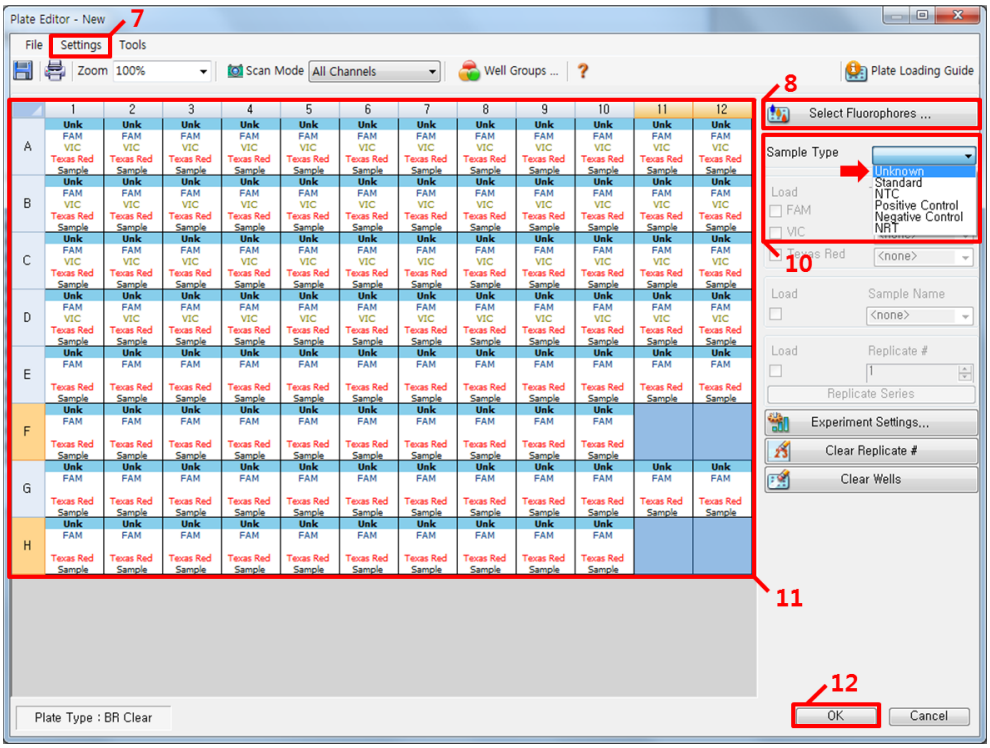
8. Enter 'Select Fluorescence'.

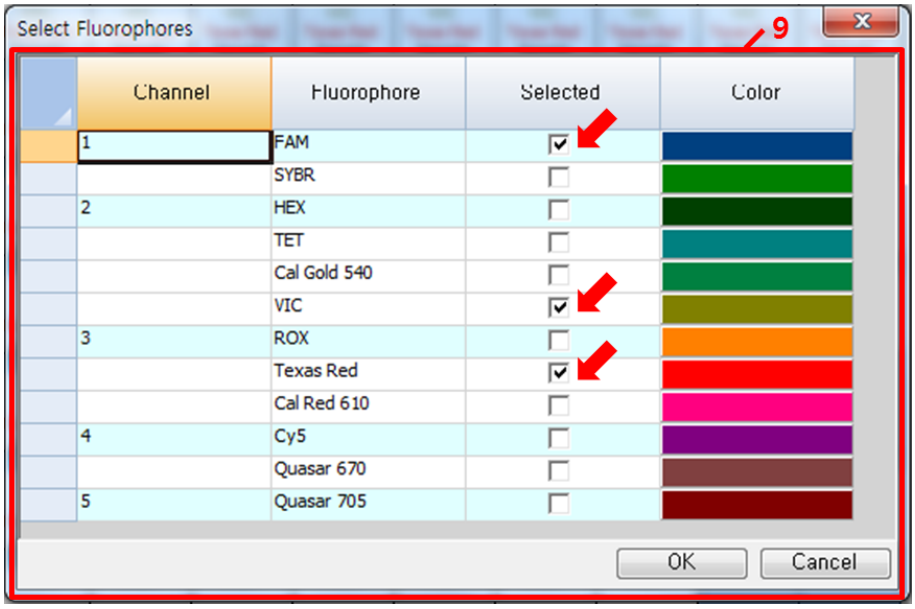
9. Select all fluorescence used in the kit (FAM / VIC / Texas Red).

10. Select Sample Type as 'Unknown'.

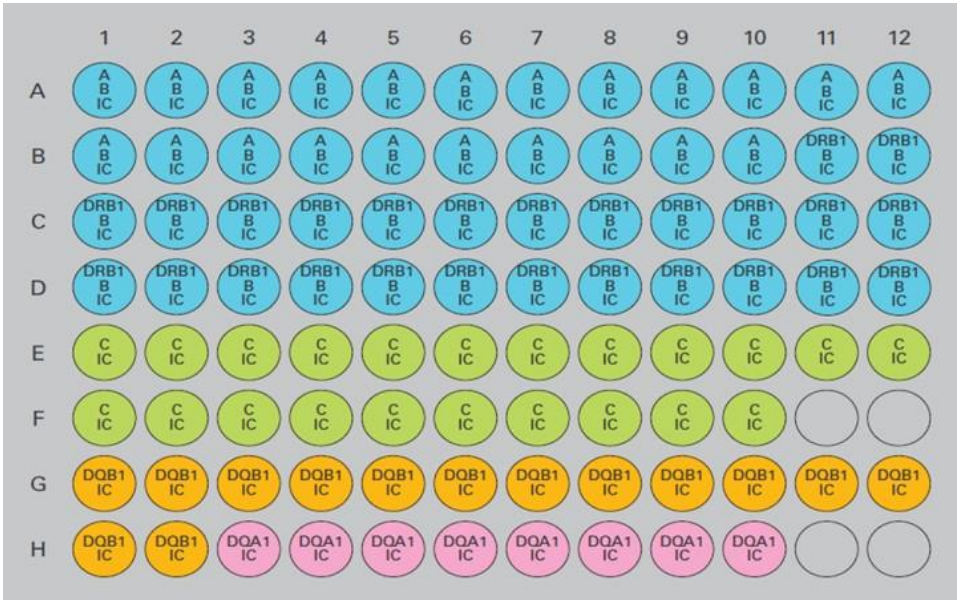
11. Choose the target position of the 'Plate Layout'. Set F11, F12, H11, H12 well to clear.

12. Click 'OK' to save the Plate File (*.pltd).



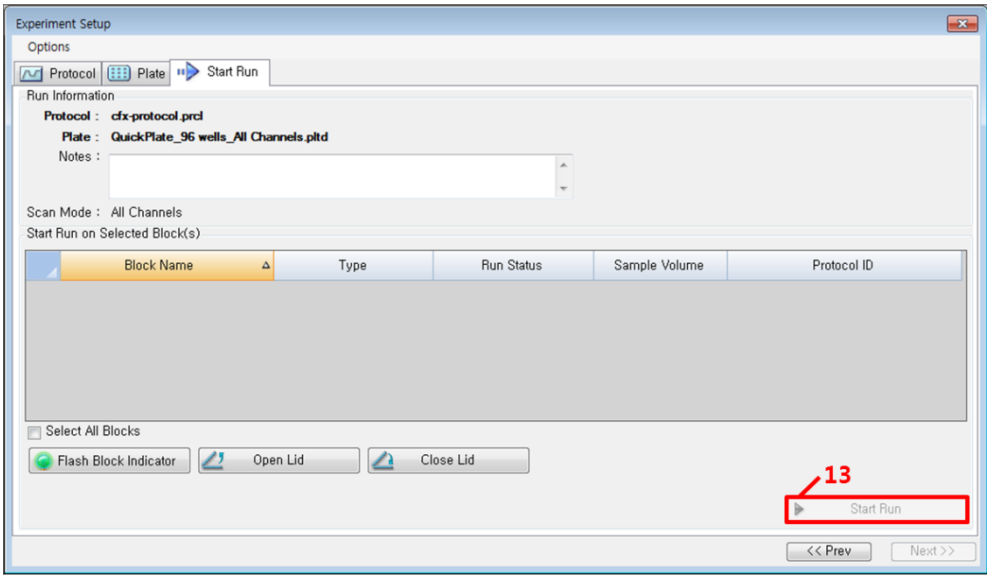


Note: The software settings should adhere to the image below, paying attention to A6, A11, B5, D11, and D12.



5> Refer to 'Experiment Setup' figure below, take things in order

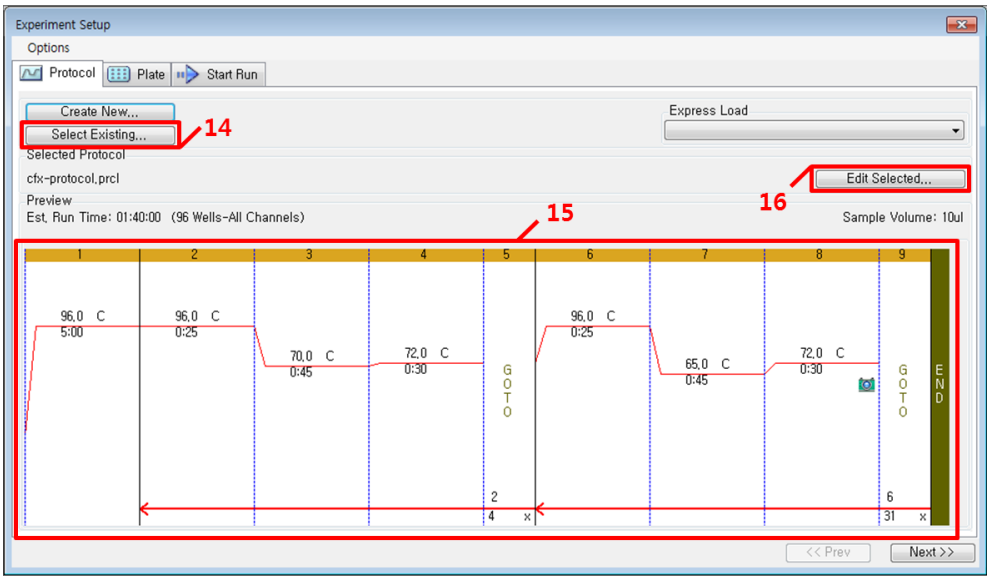
13. Insert the plate into the instrument and press 'Start Run'.



II. Saved Template Importing

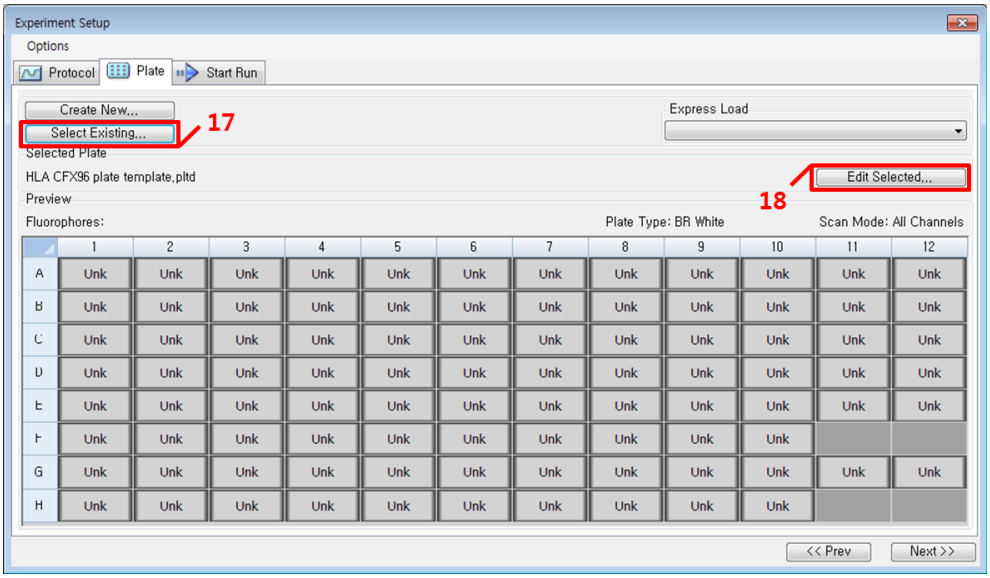
1> Refer to 'Experiment Setup' figure below, take things in order

14. In 'Select Existing…', load the Bio-Rad PCR Protocol File.
15. Check the PCR protocol.
16. If you need to modify it, go to 'Edit Selected…' and edit it.



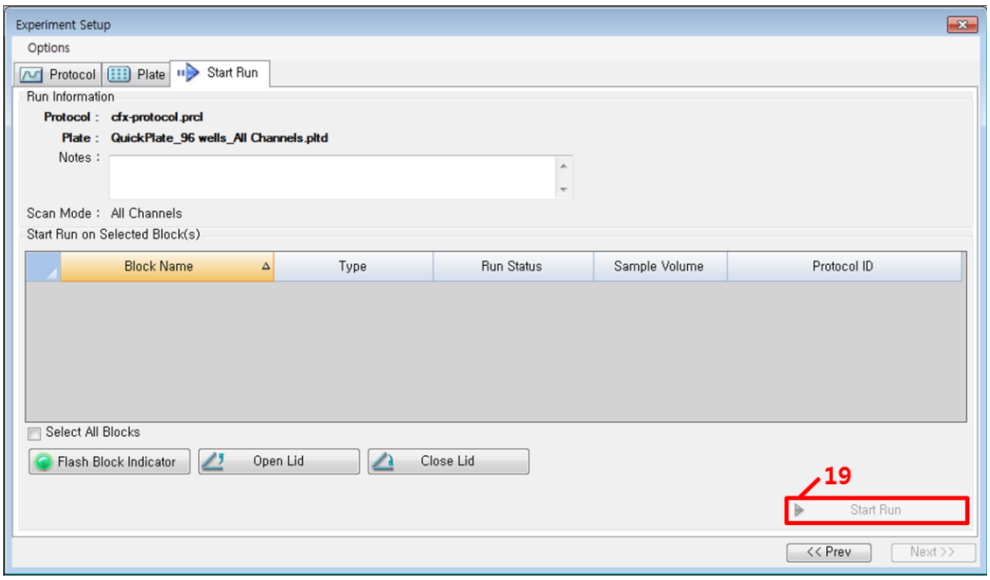
3> Refer to 'Experiment Setup' figure below, take things in order

17. In 'Select Existing…', load the Bio-Rad PCR Plate Setup File.
18. Click 'Edit Selected…' to enter the 'Plate Editor', apply the sample name and confirm the settings.



4> Refer to 'Experiment Setup' figure below, take things in order

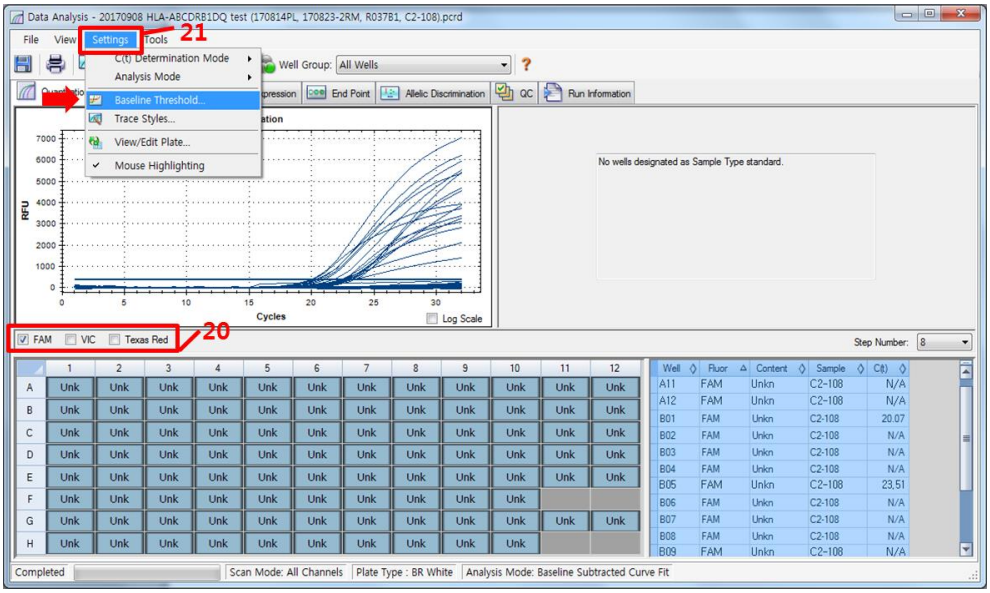
19. Insert the plate into the instrument and press 'Start Run'.



III. Analysis Setting

1> Refer to 'Data Analysis' figure below, take things in order

20. Select one by one the FAM, VIC, Texas Red respectively to set the analysis conditions.
21. Set Baseline Threshold in 'Settings'.
22. Select 'User Defined' to set the baseline.
23. Input the baseline according to '**Table 16**'.
24. Input the threshold according to '**Table 16**'.
25. Click 'OK' to apply settings.



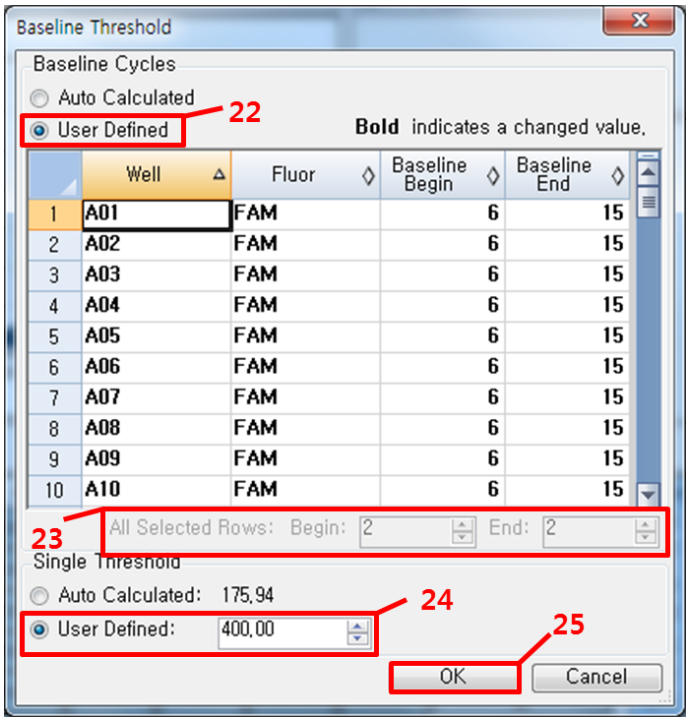


Table 16. Analysis setting for CFX96

Real time PCR	Target	Threshold	Baseline	
			Start	End
CFX96™	FAM (HLA-A, -DRB1, -C, -DQB1, -DQA1)	400	6	15
	VIC (HLA-B)	500		
	Texas Red (HLA-IC)	200		

8. RESULT INTERPRETATION

8.1 Data Analysis

1) Signals are detected in fluorescence channel FAM, JOE (VIC) and ROX (Texas Red) by ABI 7500 Fast /ABI7500 /QuantStudio™ 5/QuantStudio™ 6/CFX96™ Real time PCR. For a single test, detection of HLA-A signal is from A1 to B10 by FAM channel, HLA-B signal is from A1 to D12 by JOE (VIC) channel, HLA-DRB1 signal is from B11 to D10 by FAM channel, HLA-C signal is from E1 to F10 by FAM channel, HLA-DQB1 signal is from G1 to H2 by FAM channel, HLA-DQA1 signal is from H3 to H10 by FAM channel, and the internal control amplifications are from entire wells by ROX (Texas Red) channel. The Ct values of each locus are calculated automatically from the threshold.

Table 17. Result Interpretation

Target	Fluorescence			Acceptable Ct range	Result	Comment
	ABI7500 Fast ABI7500	QuantStudio™ 5 QuantStudio™ 6	CFX96™			
A	FAM	FAM	FAM	16~28	Positive	Results interpret as positive when Ct value is within the acceptable range
B	JOE	VIC	VIC	16~28	Positive	
C	FAM	FAM	FAM	16~28	Positive	
DRB1	FAM	FAM	FAM	16~28	Positive	
DQB1	FAM	FAM	FAM	16~28	Positive	
DQA1	FAM	FAM	FAM	16~28	Positive	
IC	ROX	ROX	Texas Red	≤32	Positive	In case of the large amount of target fragment, signal of IC can be decreased or does not appear due to PCR competition. In this case, IC and target positive reactions are acceptable.

2) Refer to the pattern chart for the specifically amplified DNA in each well. The presence of the allele- or group-specific amplification is detected as positive for the particular reaction. On the other hand, the absence of the allele- or group- specific amplification is detected as negative.

3) The internal control amplification may be weaken or not shown when the allele- or group-specific amplification is present. The presence of the specific amplification for internal control is also an indication to the success of the PCR reaction. This is not a limitation to the test.

4) The internal control amplification reaction should always be shown in negative wells. Absence of both internal control and allele- or group-specific amplification indicates failed reaction and invalid result.

5) Document the positive reactions for the test and determine the HLA types by using the pattern chart provided.

9. QUALITY CONTROL

Each lot of the kit is strictly tested for quality. The manufacturer inspects samples of each lot against a defined panel of reference DNA samples (ex-Class I, Class II UCLA DNA Reference Panel) in order to check the lot-specific variation. The alleles carried by these DNA samples react with the corresponding primer & probe mixes in the Kit. The report is available upon request.
You can also verify the product's performance using the UCLA DNA Reference Panel or DNA Sample that knows HLA typing result.

10. LIMITATION OF PROCEDURE

- This kit must be performed by well-trained and authorized laboratory staff.
- Any diagnostic result generated must be interpreted in conjunction with other clinical or laboratory findings. It is the user's responsibility to validated system performance for any procedures used in their laboratory which are not covered by the OSANG Healthcare performance studies.
- This kit should be used the biological samples such as human genomic DNA extracted by appropriate methods.
- This kit does not provide inhibition effect data obtained by medicines.
- Do not use the heparin which can interfere severely with PCR reaction.
- This kit is not a companion diagnostic device.
- If a serious incident occurs related to this device, contact the legal manufacturer and the Competent Authority.
- Performance of the test requires highly controlled test conditions to ensure adequate discriminatory amplification. The procedure described in "Instructions for Use" must be strictly followed.
- All instruments and equipment must be calibrated according the manufacturer's instructions.
- GeneFinder™ HLA-ABCDRB1DQ RealAmp Kit is optimized for the HLA-ABCDRB1DQ pol. (Taq polymerase). The use of other brands of Taq polymerase may cause false negative or positive results.
- Depending on the quality and quantity of the DNA used, the amplifications would be affected. The DNA should have an A260/A280 ratio between 1.6 and 2.0 to obtain optimal amplification.
- GeneFinder™ HLA-ABCDRB1DQ RealAmp Kit cannot resolve all allele combinations. When an ambiguous result obtained, please refer to section 11-TROUBLESHOOTING for possible causes and solutions. If the ambiguous results are still not resolved, please use a typing Kit with the same or higher level of resolution from other suppliers to resolve the ambiguity.
- The results obtained from the GeneFinder™ HLA-ABCDRB1DQ RealAmp Kit should not be used as the sole basis for making clinical decisions.
- Recommended DNA concentration is between 50 to 100 ng per $\mu\ell$. Use of not recommended DNA concentration sample may cause the inaccurate results.
- In the typing results of HLA-B, there are wells that amplify according to the type of HLA-A or C. Details are given in the pattern chart.
- When the HLA-DQB1 sample type is *06:01, the IC fluorescence signal of well No. 86 does not appear.

11. TROUBLESHOOTING

Problems	Causes	Solution
No fluorescent signal	Error in the PCR condition when setting up the PCR program	Verify the PCR cycling program
	Omitted components	Verify each component, and repeat the PCR mixture preparation
	Incorrect detector manager	Verify the detector manager
Weak the fluorescent intensity or absence of amplification	Poor DNA quality	Repeat DNA purification and make sure the A260/A280 ratio is between 1.6-2.0.
	Insufficient amount of DNA	Make sure the DNA concentration is within the suggested range of 50-100ng/㎕.
	Insufficient *Taq* polymerase activity	Make sure there is sufficient amount of *Taq* polymerase added and the *Taq* polymerase has not been degraded.
	Error in the volume of some component added in the reaction	Verify each component, and repeat the PCR mixture preparation
	Not enough volume of DNA samples added	Repeat the PCR reaction using the correct volume of DNA samples
	Thermal cycler is not working in a proper way.	Calibrate the thermal cycler and check the PCR program. A thermal cycler used for routine typing should be calibrated every 6-12 months.
If the diverse intensity of fluorescent signals appears	Use of non-calibrated pipettes.	Calibrate all pipettes routinely according to the supplier's recommendations.
	Pipetting error	Make sure that the equal volume of reactants are added in each tube or plate
	Contamination in the outer surface of PCR tubes or plate	Wear gloves during the experiment
	Incomplete centrifugation of tubes or plate	Centrifuge (2000 rpm x 2min.) plate after all reagents are added
Presence of false positive amplification	Overly high DNA concentration	Make sure the DNA concentration is within the suggested range of 50-100ng/㎕.
	Excessive *Taq* polymerase activity	Make sure the amount of *Taq* polymerase added is as instructed on page 8.
	Unusual amplification in one of Target B	Check HLA-A or C type that affect whether the B result.
	Improperly calibrated thermal cycler	Calibrate the thermal cycler
Failure of one or several PCR amplifications (control and specific amplifications)	Pipetting error	Make sure that the equal volume of reactants are added in each tube or plate
	Uneven volume of DNA-Master mixture has been added to the wells.	Perform pipetting more carefully.
	If one or two wells fail the PCR amplifications, check whether it affects typing result. If yes, the test must be repeated. If the missing wells don't affect typing result, no further tests need to be performed.	

12. PERFORMANCE CHARACTERISTICS

12.1 Analytical Sensitivity*

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit was performed for analytical sensitivity using 3 reference DNA panel (UCLA Immunogenetics Center, USA) with different concentration and tested for 7 times with each concentration. The outcome of the test was 25 ng/㎕ for analytical sensitivity.

* Note : The selection criteria for the samples used in this experiment are as follows.

i. Because there are a lot of tests, samples with a large volume of samples are preferentially selected.

ii. Samples with a high frequency of alleles rather than rare alleles were selected.

12.2 Analytical Specificity§

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit was performed for analytical specificity using 44 reference DNA panel (UCLA Immunogenetics Center, USA). The outcome was compared with defined typing results provided UCLA Immunogenetics Center, and there was 100% concordance (44/44) for the all of HLA-A, B, C, DRB1 and DQ(DQB1, DQA1) allele typing results.

HLA Locus	No. of DNA samples	No. of HLA Allele	Concordance rate
HLA-A	44	88	100%
HLA-B	44	88	100%
HLA-C	44	88	100%
HLA-DRB1	44	88	100%
HLA-DQB1	44	88	100%
HLA-DQA1	44	88	100%

§ Note : In the Analytical Test, samples were selected that contain all the types that can be identified at the low resolution level of the HLA-A, -B, -C, -DRB1, -DQB1, and -DQA1 locus of the "UCLA reference panel".

i. Untested type list: A*43, B*59, B*67, B*78, B*82, B*83

ii. B*59 and B*67 are confirmed in Diagnostic Performance Test

12.3 Reproducibility

3 of UCLA HLA Reference DNA samples were tested using GeneFinder™ HLA-ABCDRB1DQ Real*Amp* Kit for the within-lot and inter-lot reproducibility studies. The data showed 100% reproducibility for HLA-A, -B, -C, -DRB1 and DQ(DQB1, DQA1) allele typing results.

12.4 Clinical characteristics

GeneFinder™ HLA-ABCDRB1DQ Real*Amp* kit was compared with SSO typing kits by testing 100 samples. There was 100% agreement (100/100) for all HLA-A, -B, -C, -DRB1, -DQB1, -DQA1 locus between the GeneFinder™ HLA-ABCDRB1DQ Real*Amp* kit and the results obtained using the SSO tests.

N	Concordance	Discordance	Total	Agreement %
	100	0	100	100
Agreement %	100	0	100	-

N; Number of samples

13. SYMBOLS USED ON LABELS

IVD	In Vitro Diagnostic Medical Device
CE 0123	This product fulfills the requirements of Directive 98/79/EC on in-vitro diagnostic medical devices
Σ	Number of tests
EC REP	Authorized Representatives in European Community
LOT	Lot or batch number
#	Model number
REF	Catalogue number
	Consult Instruction For Use
	Caution
	Store below temperature shown
	Expiry date
	Manufacturer

14. REFERENCES

1. Gersuk VH, Nepom GT. A real-time PCR assay for the rapid identification of the autoimmune disease-associated allele HLA-DQB1*0602. Tissue Antigens 2009 73(4):335-340.
2. Terasaki PI, Bernoco D, Park MS, Ozturk G, Iwaki Y. Microdroplet testing for HLA-A, -B and –D antigens. American Journal of Clinical Pathology 1978 69:103-120.
3. Gersuk VH, Nepom GT. A real-time PCR approach for rapid high resolution subtyping of HLA-DRB1*04. J Immunol Metholds 2009 73(4):335-340.
4. Olerup O, Zetterquist H. HLA-DR typing by PCR amplification with sequence specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantations. Tissue Antigens 1992:39(5):225-235.

15. CUSTOMER SERVICE CONTACT INFORMATION

OSANG Healthcare Co., Ltd.
132, Anyangcheondong-ro, Dongan-gu, Anyang-si, Gyeonggi-do, 14040, Korea
Tel: +82-31-460-0300 / Fax: +82-31-460-9933 / Web site: www.osanghc.com

Technical Support

Tel: +82-31-460-0472

16. REVISION HISTORY

REVISION	DATE(YYYY-MM)	CHANGES
0	2018-05	New Issue for IVDD Regulation
1	2018-08	Revision of PROCEDURE
2	2019-01	Add reference number
3	2022-08	Revision of INTENDED USE Revision of storage temperature condition
4	2022-09	Revision of storage temperature condition
5	2024-04	Additional 2. INENDED PURPOSE Additional 3. TEST PRINCIPLE Additional 6. WARNINGS AND PRECAUSIONS Revision of 7. PROCEDURE Additional 10. LIMITATION OF PROCEDURE Additional 17. REVISION HISTORY