BIO-RAD

CFX Real Time PCR System

Instrument Guide

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CFX Maestro software user guide

The CFX series is operated with CFX Maestro software.

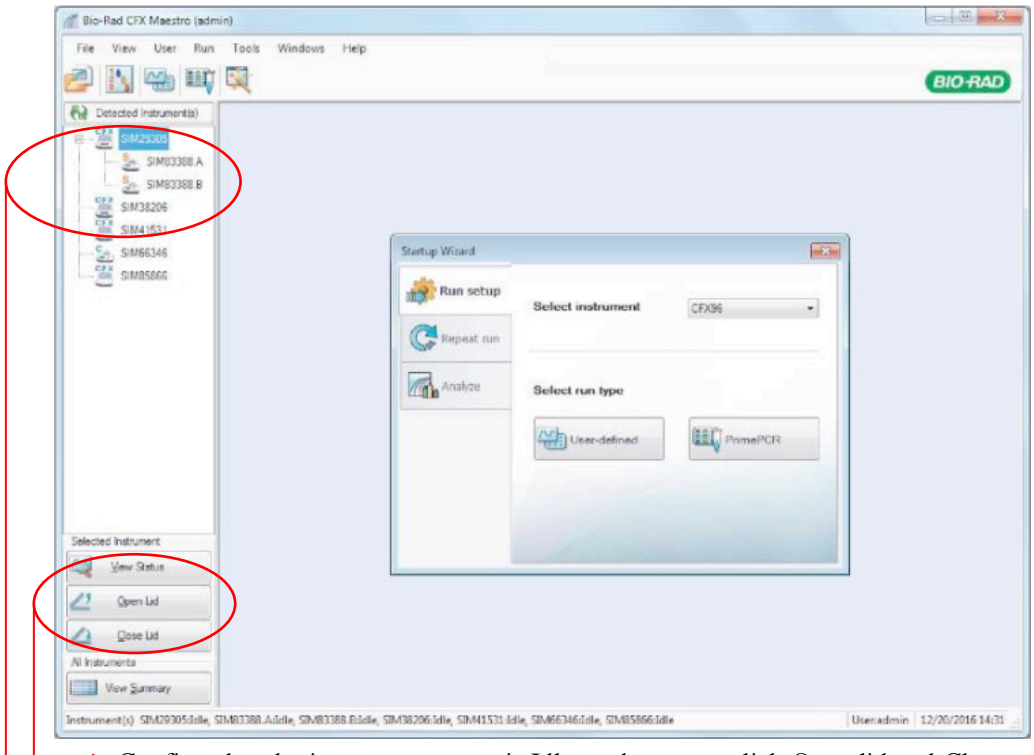
It is recommended to install on Windows 10 (64-bit) for operation.

Software operation

- ✓ **After connecting to the instrument, switch on the computer at first and power on the instrument. Then, click and initiate software. Notification: After the detection was finished, turn off the instrument in reverse order.**
- ✓ **Confirmation of the software connection**

1. Checking the connection status
2. Instrument can be operated via 「open lid」 as well as 「close lid.」 （Fig.1）

Fig.1



Confirm that the instrument status is Idle, and you can click Open lid and Close lid to control the instrument to open and close the lid.

The instrument serial number will be displayed after the software is connected.

- ✓ **Click User-defined in Startup Wizard to set the experimental protocol (Fig. 1)**

Three step for setting program <Protocol→Plate→Start Run>

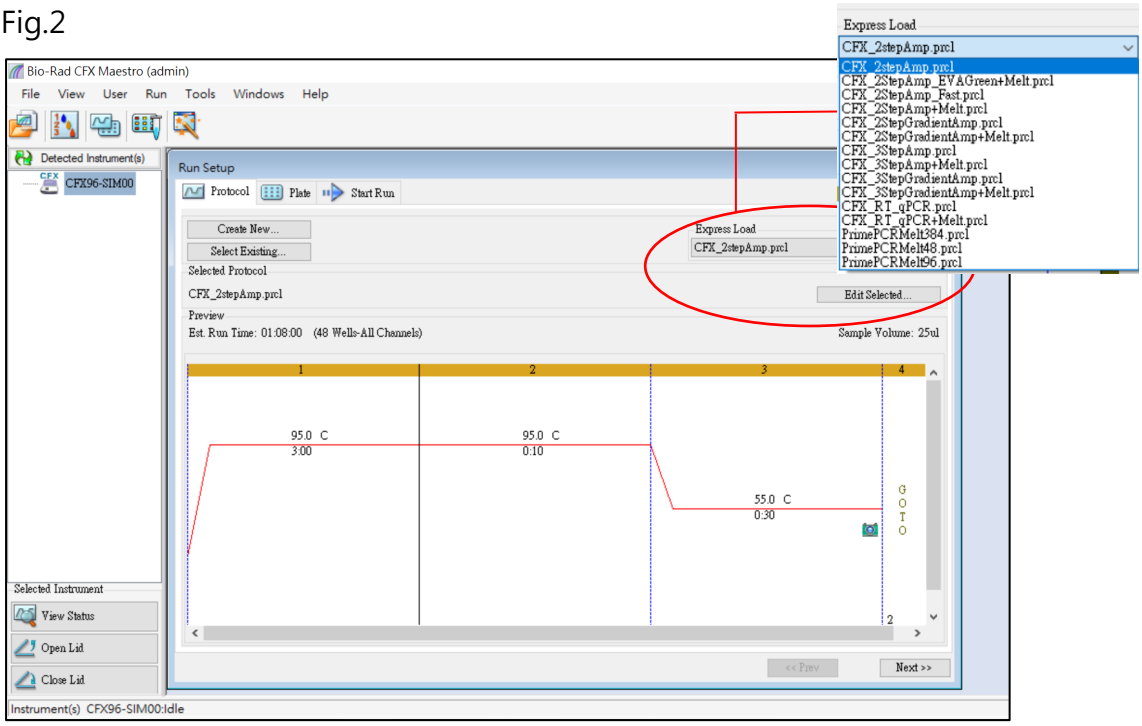
✓ **Step1. Set up the protocol，Adjust reaction temperature and time base on experimental design.**

1. Select 「Express Load」

   Notification: Commonly used protocols have been built in the software (Fig.2)

2. Select a built-in protocol template, then click 「Edit Selected」at below to perform edition or modification (Fig.2)

   Fig.2

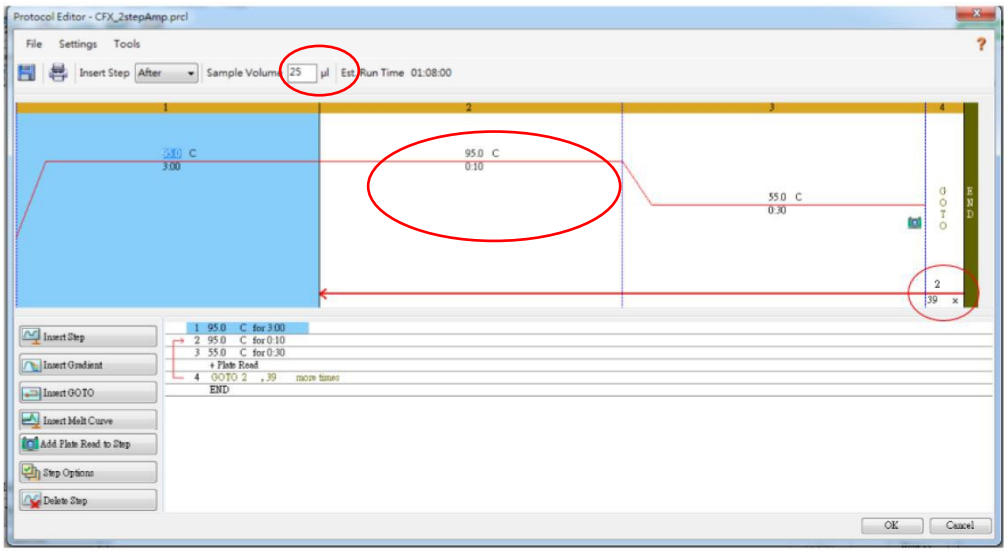


3. Protocol edition and modification

   - Reaction temperature, reaction time and sample volume can be directly edited and modified (Fig.3)
   - Cycle numbers are able to be modified through 「 GOTO 」, which indicates anticipatable cycle numbers must to be 「- 1」. (Fig. 3)

   . (Ex: 40 cycles will key in 39)

Fig.3



Protocol setting can be adjusted on left toolbar menu at the bottom of window (Fig.4).

Fig.4

Insert Step: inserts a step before or after the selected

Insert Gradient: inserts a gradient step based on the type of well block

Insert GOTO : inserts a cycling (loop) step

Insert Melt Curve : inserts a melt curve read

Add Plate Read to Step : adds plate read command to the selected step. **

Step Options : displays the options available for the selected step

Delet Step: deletes the selected step from the protocol.

** Tip: After you add a plate read command to a step, the button changes to Remove PlateRead when you select the step.

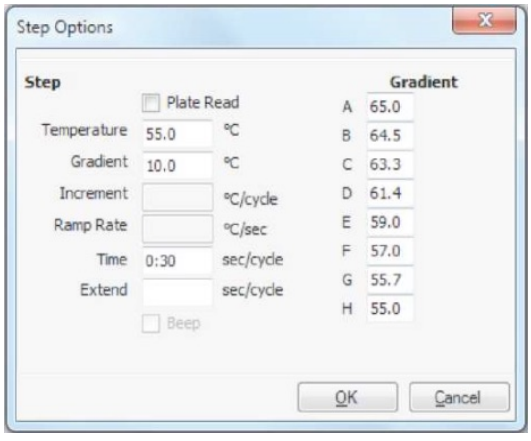
Fig.5

Sets the target temperature

Sets the gradient range (1–24°C)

To increase (or decrease) the temperature

The ramp rate for the selected step

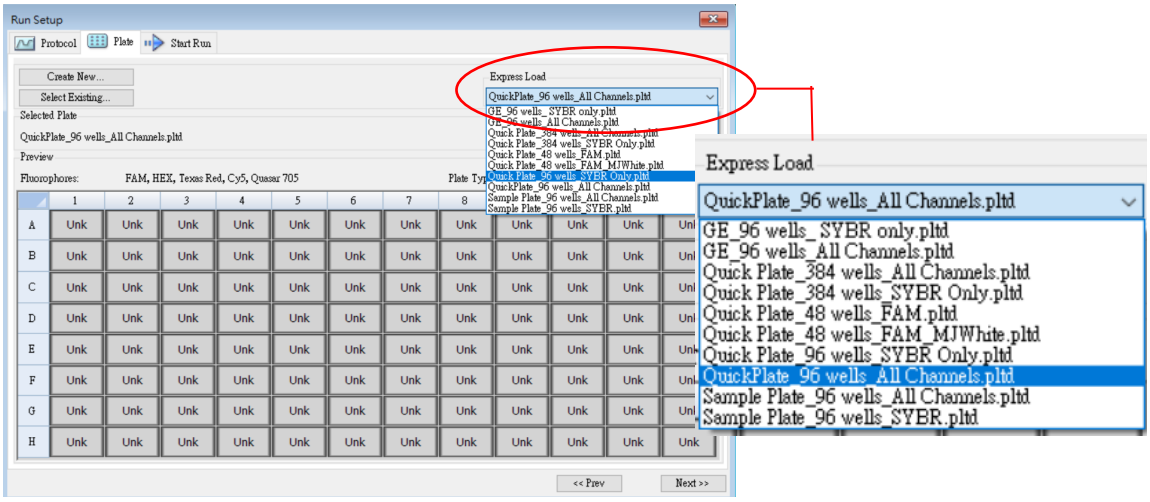


• After modification of the temperature time step, save it and click 「Next」 .

✓ **Step2. Select fluorescent channel module**

On the 「Plate」tab site, click "Express Load", choose a built-in protocol and select the corresponding fluorescent channel module for the experiment. (Fig.6)

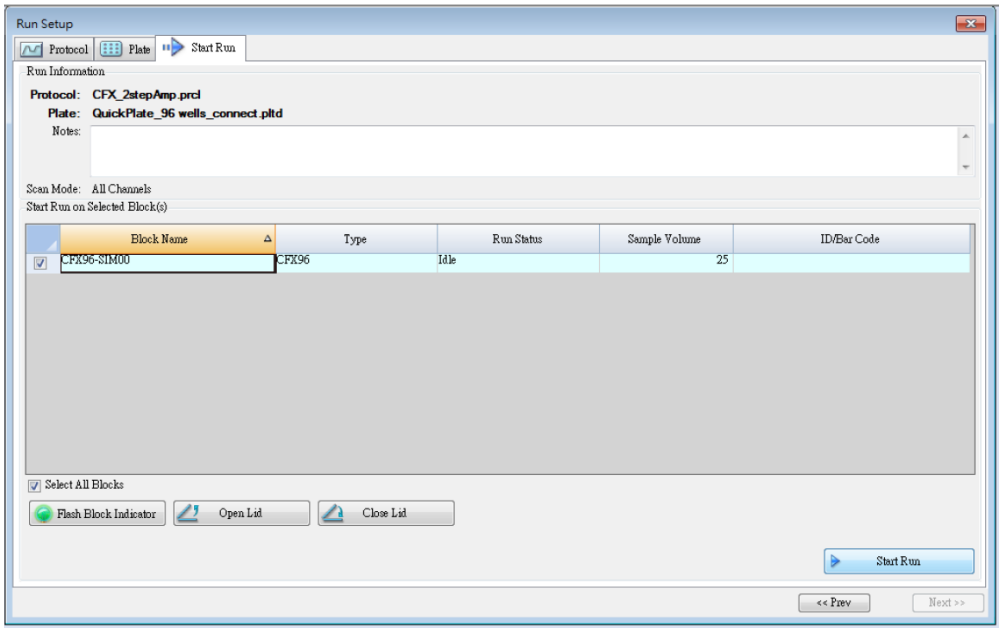
Fig.6



✓ **Step2. : Start Run**

- After confirming the serial number, status and volume of the instrument. Then, you can click 「Start Run」 to start the experiment (Fig.7)
- Save the experimental data in personal folder and the analysis data will be label with "pcrd" file.

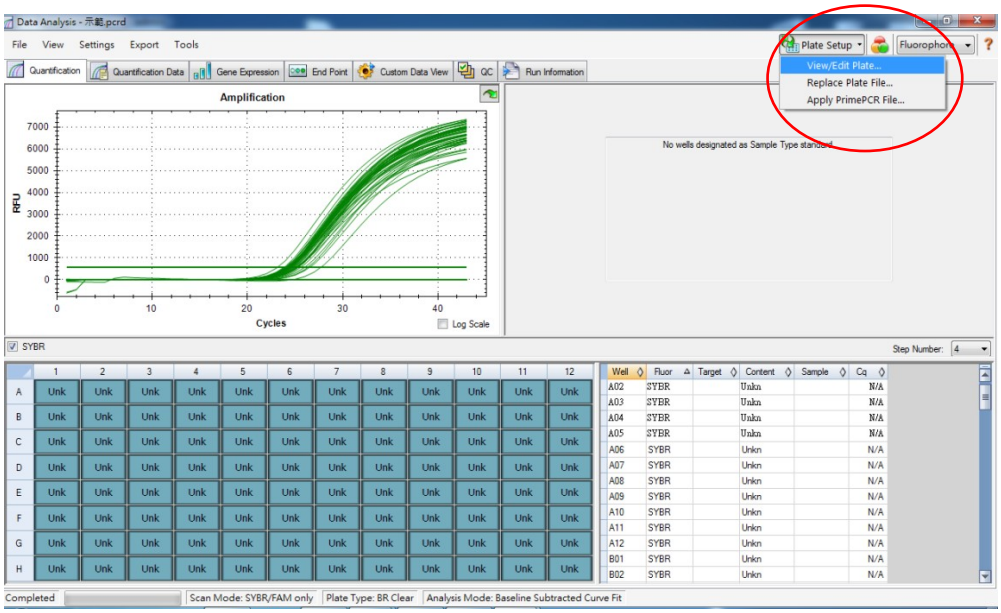
Fig.7



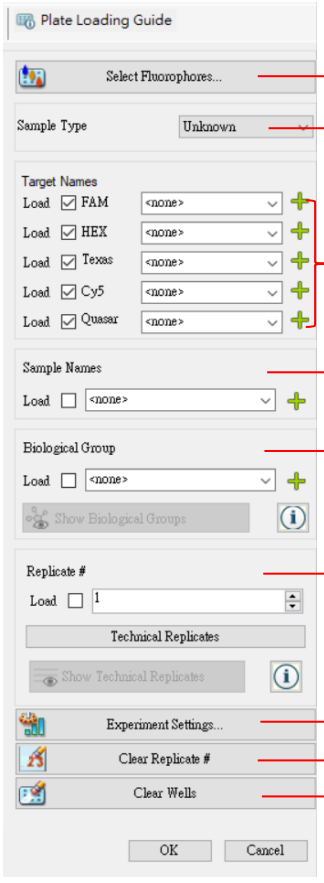
Data analysis

✓ **After the experiment was finished, Click on 「Plate Setup」 on the upper right page and select「 View Edit Plate」 (Fig. 8) to enter into the Plate Editor. On the page of the plate, select the area at first and input or edit the sample name stepwisely from right to left.**

Fig.8



圖九



Select the requisite fluorophores for the plate:

Select sample type (unknown, standard, NTC, PC,

Targets of interest (genes or sequences) in each loaded well.

The identifier or condition that corresponds to the sample.

The identifier or condition that corresponds to a group.

Enter repeatable sample settings (Horizontal / Vertical)

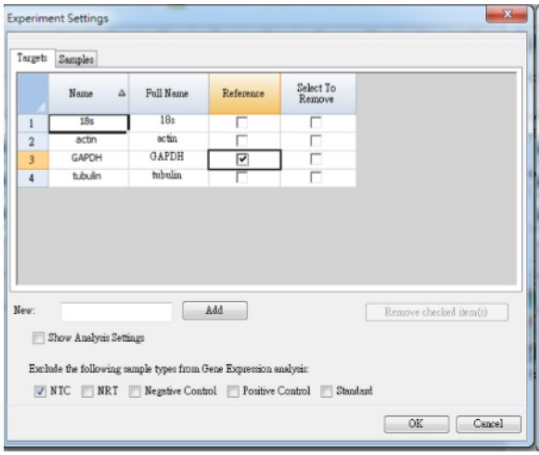
Setting reference gene and control sample (Fig.10)

Remove all of Replicate

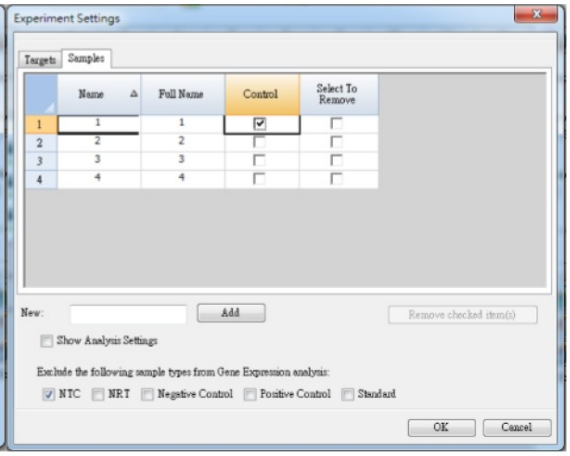
Clear all well

Fig.10

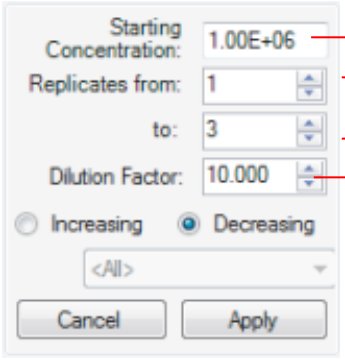
選擇 Reference gene



選擇 Control Sample



** When the sample is spiked with a standard of known concentration, enter the reaction concentration



The concentration value from which the series starts

The replicates in the series to which the dilution factor will be applied

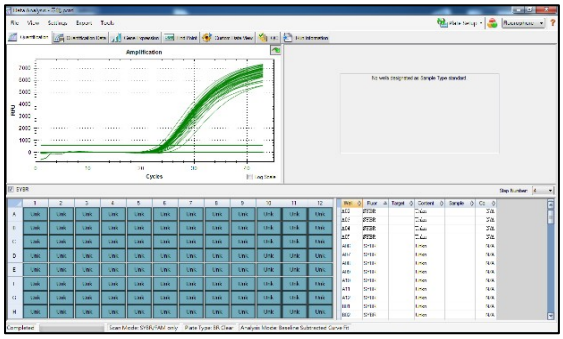
The amount to change the concentration within each replicate

✓ **Press「ok」 to process the data analysis page after setting the Plate.**

✓ Data Analysis and Application tool

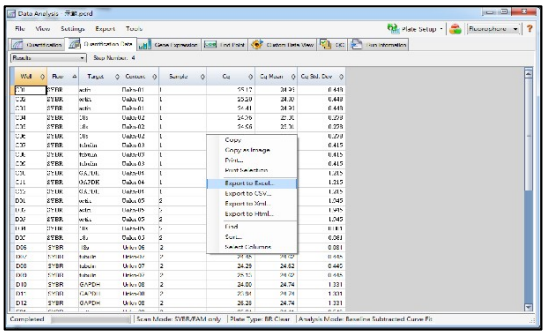
Quantification

Amplification curves can be observed along with their Cq values



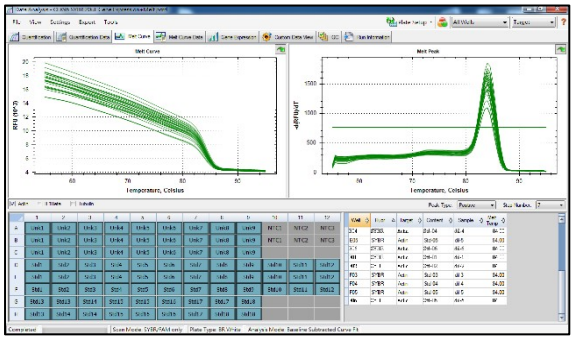
Quantification Data

The raw data for this experiment is presented, including thresholds and mean values for each sample and standard deviation of replicates.



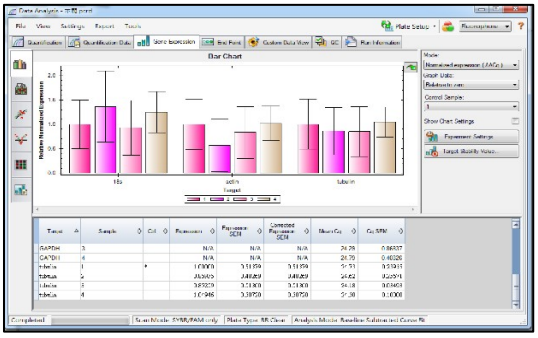
Melt Curve Data

Observe the melting curve and its Tm value



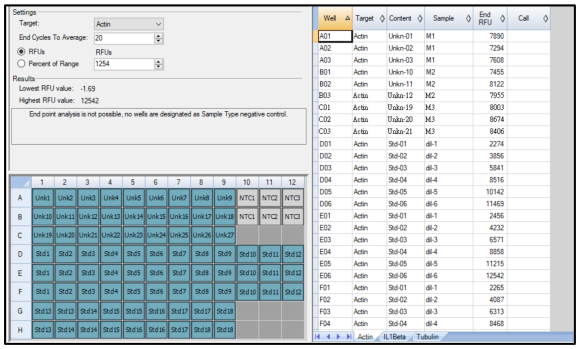
Gene Expression

Observe the relative expression of genes



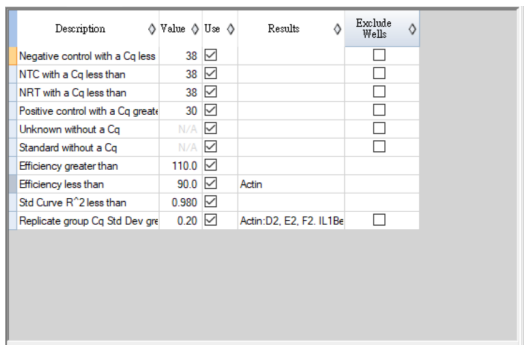
End Point Analysis

Present data analysis data of the end point



Quality Control

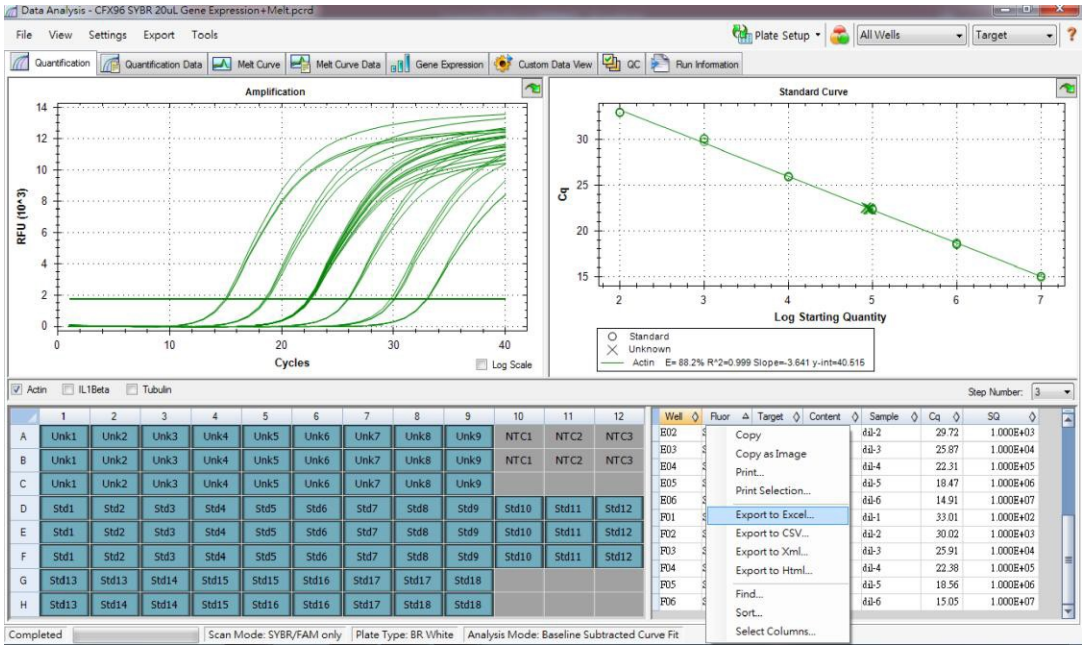
Provide evaluation information



Description	Value	Use	Results	Exclude Wells
Negative control with a Cq less	38	☑		☐
NTC with a Cq less than	38	☑		☐
NRT with a Cq less than	38	☑		☐
Positive control with a Cq greate	30	☑		☐
Unknown without a Cq	N/A	☑		☐
Standard without a Cq	N/A	☑		☐
Efficiency greater than	110.0	☑		
Efficiency less than	90.0	☑	Actin	
Std Curve R^2 less than	0.980	☑		
Replicate group Cq Std Dev gre	0.20	☑	Actin:D2, E2, F2. IL1Be	☐

Save file

To save Excel, right-click and select「 Export」 for Excel form. If you want to save image file, you can choose 「Save Image」 as to save it as a JPG file.



Output as PDF

Select 「Report 」in Tools on the upper toolbar. You can customize the report and save as a PDF file.