

CFX Real Time PCR System

Instrument Guide





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 \lceil open lid \rfloor as well as \lceil close lid. \rfloor (Fig.1)

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Confirm that the instrum	nent status is Idle, and you	can click Open lid and Close
	ment to open and close the	lid.

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 $\ ^{\Gamma}$ Edit Selected _ at below to perform edition or modification (Fig.2)

Fig.2		Express Load	
		CFX_2stepAmp.prcl	~
Image: Start Start Run Select Existing	Express CFX_2n abb)	CPK 25kpAmp prel CPK 25kpAmp EVAGreen+Meltprel CPK 25kpAmp Fastprel CPK 25kpAmp Fastprel CPK 25kpAmp Fastprel CPK 25kpAmp prel CPK 25kpAmp prel CPK 25kpAmp prel CPK 25kpAmp prel CPK 25kpPredentAmp prel CPK 25kpP	
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Close Lid		<< Prev Next >>	
Instrument(s) CFX96-SIM00:Idle			

- 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 \lceil GOTO $_{
 m J}$, which indicates anticipatable cycle numbers must to be $\lceil -1 \rfloor$. (Fig. 3)
 - . (Ex: 40 cycles will key in 39)





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

Fig.4

Insert Step
[1] Insert Gradient
Insert GOTO
Insert Melt Curve
Add Plate Read to Step
Step Options
🙀 Delete Step

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.

Fig.5

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

ets the target temperature ets the gradient range (1–24°C) to increase (or decrease) the temperature the ramp rate for the selected step $\frac{5 \text{tep}}{2 \text{ Plate Read}} + A = 65.0 \text{ ec} + B = 64.5 \text{ gradient} = 10.0 \text{ ec} + C = 63.3 \text{ gradient} = 10.0 \text{ gradient} = 10.$		Step Options				
	Sets the target temperature Sets the gradient range (1–24°C) To increase (or decrease) the temperature The ramp rate for the selected step	Step Temperature Gradient Increment Ramp Rate Time Extend	Plate F 55.0 10.0 0:30	Read °C °C/cycle °C/sec sec/cycle sec/cycle	A B C D E F G	Gradient 65.0 64.5 63.3 61.4 59.0 57.0 55.7 55.0

- After modification of the temperature time step, save it and click $\ \ \$ Next $\ \ \$.

✓ Step2. Select fluorescent channel module



✓ 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				
Run Setup				
M Protocol 💷 Plate 🕩 Start Ru	n			
Run Information				
Protocol: CFX_2stepAmp.prcl				
Plate: QuickPlate_96 wells_con Notes:	nnect.pltd			
				<u> </u>
Soon Mode: All Channels				v
Start Run on Selected Block(s)				
Block Name	△ Type	Run Status	Sample Volume	ID/Bar Code
CFX96-SIM00	CFX96	Idle	25	
Select All Blocks				
Flash Block Indicator	en Lid 🛛 💦 Close Lid			
				Start Run
				<< Prev Next >>

Data analysis

✓ After the experiment was finished, Click on ^Γ Plate Setup 」 on the upper right page and select ^Γ 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

職 Plate Loadin	ıg Guide		
ta Se	lect Fluorophores		- 9
Sample Type	Unknown	n 	- 9
Target Names			
Load 🖂 FAM	<none></none>	~ 🕂	
Load 🔽 HEX	<none></none>	~ +	
Load 🖂 Texas	<none></none>	~ + -	
Load 🔽 Cy5	<none></none>	~ +	
Load 🗹 Quasar	<none></none>	~ +	
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Show Tech		i	
👬 Ез	cperiment Settings		_
A	Clear Replicate #		_
E	Clear Wells		_
	OK	Cancel	

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
Experiment Settings	Experiment Settings
Name A Full Name Reference Select To Remove 1 35s 10s Image: Constraint of the second seco	Name Pull Name Control Salet To Remove 1 1 I <
New: Add Remove checked (tem(s) Show Analysis Settings Exclude the following sample types from Gene Expression analysis: VITC NRT Negative Control Positive Control Shanlard OK Cancel	New: Add Remove checked them(d) Chow Analysis Settings Exclude the following sample types from Gene Expression analysis: V NTC NR.T Negative Control Ponitive Control Standard OK Cancel

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

Starting Concentration: 1.00E+06	The concentration value from which the series starts
Replicates from: 1 (*) to: 3 (*)	The replicates in the series to which the dilution factor will be applied
Dilution Factor: 10.000	The amount to change the concentration within each replicate
Increasing Occreasing	
<al></al>	
Cancel Apply	

✓ Press rok_{J} 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.

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Melt Curve Data

Observe the melting curve and its Tm value



End Point Analysis

Present data analysis data of the end point



Gene Expression

Observe the relative expression of genes



Quality Control

Provide evaluation information

Negative control with a Cq less 38	Description 👌	Value 👌	Use	٥	Results 👌	Exclude Wells	0
NTC with a Cq less than 38 ✓ □ NRT with a Cq less than 38 ✓ □ Positive control with a Cq great/ Unknown without a Cq 30 ✓ □ Standard without a Cq N/A ✓ □ Bridency greater than 100 ✓ □ Bridency greater than 900 ✓ Actin Sta Gunre R°2 less than 0.980 ✓ □ Replicate group Cq Sd Dev gr 0.20 ✓ Actin:D2, E2, F2, IL, IBE □	Negative control with a Cq less	38	\square				
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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



Completed Scan Mode: SYBR/FAM only Plate Type: BR White Analysis Mode: Baseline Subtracted Curve Fit