Real-Time Quantitative PCR System Isothermal PCR Analyzer

LTrtPCR1-X4/5 LTisPCR1-X4

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For Research Use Only. Not for use in diagnostic purpose.

Please read this manual carefully before using the instrument and fully understand the precautions.

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SAFETY INFORMATION

- Please read and fully understand the following safety precautions.
- Please operate the instrument strictly in accordance with the operating instructions of this user manual to ensure safety.
- The safety instructions in the user manual are explained. The operations or matters shown in "WARNING", "CAUTION" and "NOTE" may cause danger or problems to the experiment, so be sure to pay attention to the operations.

WARNING:

This **WARING** indicates that any operation or use, if not strictly followed the user manual, may result in personal injury or instrument damage.

- Never operate the instrument without the ground connected.
- End users are not allowed to disassemble the plastic casing of the instrument, replace components or adjust the instrument, and it is strictly forbidden to disassemble the instrument under power-on conditions. If necessary, please contact professional after-sales engineers for instrument maintenance and repair.
- Use supplied power cord for the instrument. If the power cord is damaged, it must be replaced with a power cord of the same type or with specification properly configured and approved.
- Do not plug or unplug the power cord when the power switch is on.
- Do not touch the hot lid when the instrument is powered on.

CAUTION:

This **CAUTION** indicates that any operation or use, if not strictly followed by the user manual, may result in damage of the instrument or wrong results.

- The instrument should be installed in a place with low humidity, less dust and away from water sources (pools, water pipes, etc.). The laboratory should be well ventilated and free from corrosive gases or strong magnetic fields. The workbench or laboratory table on which the instrument is placed should be stable.
- The vents under the instrument and on the back of the instrument are designed for the ventilation and cooling of the instrument. To ensure the normal operation of the instrument and accurate experimental data, these vents must not be blocked or covered.
- High ambient temperature will affect the test performance of the instrument. The instrument should be kept away from heaters, hot stoves and all other heat sources. Do not use the instrument in the direct sunlight or strong light, so as not to affect the reliability of the fluorescence detection of the instrument.

- When the instrument is not in use for a long time, please cut off the power.
- Avoid liquid spilled onto/into the instrument.
- After the run, the consumables should be removed from the instrument. Consumables should not be left in the instrument for a long time.

NOTE:

This **NOTE** indicates a section or content of special concern, emphasizing common errors in the functionality, operation, or maintenance of the product.

- If the following situations occur, please cut off the power immediately, unplug the power cord, and contact the Service Support of supplier:
- Liquid spilled/dropped into instrument.
- Any abnormal sound or smell appears after the instrument is powered on.
- The instrument has been accidentally dropped or the casing has been damaged.
- Instrument performance has changed significantly.
- Do not operate the instrument in any way that is not instructed or described in the user manual. If you suspect a problem, contact the manufacturer.
- The descriptions in this guide attempt to cover all possible operational risk indications. But be aware of the unexpected problems, please contact the manufacturer.

CHAPTER1 PRODUCT INTRODUCTION

1.1 Instrument Introduction

1.1.1 Description of the Instrument

LTrtPCR1-X4/5 is an easy to use 16-wells Real-Time Quantitative PCR system. The instrument is equipped with a 7-inch color touch screen, which can be operated independently. The small footprint of platform and ultra-fast ramping speed make it ideal for point-of-care and on-site testing applications.

The instrument can be configured to 2-channels, 4-channels, and 5-channels for broad applications.

1.1.2 Features of the Instrument

1. Based on confocal scanning fluorescence detection technology, it can effectively remove the interference of background light and excitation light, obtain fluorescent signals with high signal-to-noise ratio, and save the use of fluorescent probes and dyes.

2. The instrument uses linear fluorescence time-sharing scanning technology to ensure the uniformity of all fluorescence acquisitions, without the need for special reagent correction, and improve the accuracy and uniformity of CT.

3. The innovatively designed spectral separation technology effectively removes cross-interference of fluorescence between channels.

4. High-performance, long-life single-color LEDs are used as excitation light sources, without thermal attenuation, and maintenance-free for life.

5. High-performance silicon PMT can obtain high fluorescence signal under weak excitation light and reduce photo bleaching.

6. The 5-color and 16-well detection time can be shorten to 2S, which can minimize the difference in PCR extension time of different wells caused by the detection time, and shorten the experiment time at the same time. The experiment with 40 cycles can be completed within 20 minutes.

7. The block is made of alloy with specially designed heat conduction, it can effectively transfer heat while improving the heating and cooling rate and maintaining the best temperature uniformity, the temperature uniformity can be controlled within the range of $\pm 0.15^{\circ}$ C.

8. The instrument uses high-efficiency gold-plated Peltier, which can provide heating and cooling operations with maximum efficiency and a heating and cooling rate of up to 10°C/s.

9. With a specially designed cooling system, it can maintain high-speed heating and cooling while achieving ultra-quiet operation, with an average operating noise of about 50

	LTrtP	CR1-X4/5	LTisPCR1-X4			
Product Type	Real-Tim	e QPCR	RT-Isothermal PCR			
	Thermal Cor	ntrol System				
Sample capacity		1	6			
Compatible consumables		0.1mL C 0.1mL Clear	lear tube · 8-strip tube			
Sample volume		10-5	0 uL			
Heating and Cooling method	Pel	tier	Heating element			
Maximum ramp rate	10.0	° C/s	1° C/s			
Temperature range	4-100 °C 30-80		30-80°C			
Temperature accuracy	+ 0.	1°C	+ 0.2°C			
Temperature uniformity	±0.1	5°C	+0.15°C			
	Optical Dete	cting System				
Excitation light	4/5	5pcs high efficien	cy single color LED			
Detector		SiP	MT			
Detection method	Time resolved real-time scanning					
Range of excitation/emission wavelengths		455-650nm/510-750nm				
Detection Channels	2/4 channels chanr	s (optional 5 nels)	4			
Supported dyes	FAM/SYB VIC/JOE/HEX/T Red, Cy5/LIZ Cy5	R Green, ET, ROX/Texas , (Optional- .5)	FAM/SYBR Green, VIC/JOE/HEX/TET, ROX/Texas Red, Cy5/LIZ.			
Multiplex	4 ta	arget genes (opti	onal 5 target genes)			
Sensitivity		1 copy	/ gene			
Resolution	Distinguish ?	1.33-fold copy nu reac	mber difference in single-plex			
Dynamic range		10 orders o	f magnitude			
Operation Method		7"touch	screen			
	Analysi	s Mode				
	Absolute quar Melting	ntification and g curve	Absolute quantification			
	Data E	Export				
original result, data and	result in excel, p	rogram setting, a	mplification curve image			
Data connection			USB			
Operation			7 touch screen			
Power	Power AC100-240V 50/60Hz					

1.1.3 Specification of the Instrument

Dimension(LxWxH)	300*260*110mm,5.7Kg
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1.1.4 Software Function

- 1. Parameter setting function (fluorescence channel, temperature, time, number of cycles, fluorescence collection point, data processing mode)
- 2. Sample information recording function (experiment time, sample ID, sample type, target information)
- 3. Test data export function (fluorescence threshold, fluorescence value, CT value, Tm value)
- 4. Data result analysis function (absolute quantification, relative quantification)
- 5. Data storage function (setting data, running data, analysis results)
- 6. Fault reminder function

1.1.5 Standard Compliance

The structure of instrument complies with the following safety standards:
EN 61010-1
EN 61010-2-020
EN 61010-2-101
The structure of instrument complies with the following electromagnetic compatibility
standards:
EN 61326-1/FCCPart15Subpart B/ IECS 001
EN 61326-2-6:2006
The instrument complied with following EU standards:
EMC guidelines:2004/108/EC
LVD guidelines: 2006/95/EC

Changes or modifications not expressly approved by the party responsible for compliance could void the manufacturer's authority to operate the equipment.

NOTE: This instrument has been tested and founded to comply with the limits for a Class A digital device, pursuant to the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the instrument is operated in a commercial environment. This instrument radiates radio frequency energy and if not installed and used in accordance with the user manual, may cause harmful interference to radio communications. Operation of this instrument in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at their own expense.

CHAPTER 2 HARDWARE OF THE INSTRUMENT

2.1 Overview of the Instrument



Power Inlet: Connect to 110V~220V power, grounding capability required.

Power Switch: Press "I" for ON, and "O" for OFF.

Data Port: Connected to PC with data cable.

CAUTION:

1. During the working process, ensure the power cord and data cable are connected reliability, and do not plug or pull the power cord and data cable.

2. No obstructions to block the ventilation within 300mm from the vents.

3. During the working process, it is forbidden to power off, unless a fault occurs and the power supply needs to be cut off on emergency.



Hot lid: The heater is located inside of the hot lid to prevent the evaporation of reagent from condensing on the reaction tube. Before the experiment, the hot lid must be pressed in place. When the experiment is completed, press the door button to open the hot lid.

Sealing ring: A flexible rubber sealing ring is surrounding the heater, which is used for closing light, heat preservation, and preventing the reagent evaporated from condensing on the reaction tube cover.

Heating block: The compatible consumable for heating block is 8*0.1mL*2 clear PCR strip tube with flat cap and 16*0.1mL clear individual tube with flat cap.



2.2 Compatible Computer

nQ(L)16T-X4/5 can be used combine with any PC or laptop with specifications as below:

Hardware:

CPU: Inter i5 or higher

- RAM: 4G or higher
- HD: 500G or higher

USB2.0 Monitor resolution: 1920*1080(fixed Software: Win10 or higher

2.3 Compatible Consumables

To achieve best performance, especially the heating and cooling rate, the LTrtPCR1-X4/5 is designed only for standard 100uL clear tubes. Please use the recommended consumables, below is one suitable consumable for LTrtPCR1-X4/5, you can also select your own consumables to fit it.

ltem	Description	Brand	Cat number	
4	0.1ml LOW PROFILE, CLEAR PCR TUBES &		040004	
1	CAPS 8 STRIP TUBES & REAL TIME CAPS	BIOWE	910001	
0	0.1ml LOW PROFILE, CLEAR PCR TUBES &			
2	CAPS 8 STRIP TUBES & REAL TIME CAPS	AXYGEN	PCR-0108-LP-RT-C	

CAUTION:

- 1. Only 0.1mL low profile PCR tube can be used.
- 2. Only clear tube or strip can be used.
- 3. Only flat cap can be used.
- 4. The shape of the tube may affect the heating and cooling rate, fluorescence gathering

and condensation of the reagent.

CHAPTER 3 INSTRUMENT INSTALLATION

LTrtPCR1-X4/5 can be installed by end user with basic training, if you encounter problem

when install the instrument, please contact supplier for help.

3.1 Preparation and Inspection

Please check carefully before unpacking, and pay attention to the following conditions:

- 1. Deformation of outer packaging or obvious signs of damage.
- 2. The outer packaging contains obvious traces of water immersion.
- 3. The outer packaging contains signs that it has been opened.
- 4. The outer packaging has signs of being opened.

The box includes below items:

ltem	Description	Unit	Quantity
1	LTrtPCR1-X4/5 Main Unit	unit	1
2	Power Cord	piece	1
3	USB2.0 Data Cable	piece	1
4	Fuse 3.15A	piece	2
5	USD disk (Driver, User software, User manual)	piece	1
6	Quick Installation Manual	сору	1
7	QC &Warranty card	сору	1

After unpack the outer box, please inspect the appearance of instrument as following items:

- 1. The instrument plastic shell has no obvious damage.
- 2. The visible metal parts of the instrument are free from scratches and rust.
- 3. The instrument and accessories are not damaged or lost.

CAUTION: If there is damage or item lost, please contact to the supplier and do not install the instrument.

3.2 Install the Instrument

3.2.1 Environmental Requirements

Parameters	Specifications
Environment	Indoor use only
Operating altitude	Up to 3,000 meters above sea level
Ambient room temperature	15°C ~ 30°C

Transport and storage temperature	-20°C ~ 60°C
Relative humidity	≤85%

1. The instrument must be installed on a solid and flat table, and the four corners of the instrument must be in contact with the table.

2. It is strictly forbidden to expose the instrument to direct sunlight.

3. The instrument should be kept away from heat sources and liquids.

4. Keep certain space around the instrument, and the back of the instrument is required to be \geq 300mm away from the wall.

CAUTION: Operation of the instrument beyond the environmental conditions described above will not guarantee the reliability of the data. If the temperature and humidity exceed the above ranges, please use indoor air conditioning equipment and avoid direct airflow to the instrument.

3.2.2 Electrical Requirements

- 1. Power voltage: 110V ~ 220V AC, 50/60Hz.
- 2. Maximum power usage 200W.
- 3. Grounding capability required.

WARNING: Improper grounding may cause electric shock to personnel or damage to the instrument.

3.2.3 Unlock the Transport Lock

The system is locked during transportation to protect the moving parts, so it must be unlocked before installation.

There are detail guides in quick installation manual for how to unlock the system.

- 1. Loosen the nut labeled with LOCK and get it off.
- 2. Fix it to the hole labeled with UNLOCK for future usage.



3.3 Load and Remove the Consumables

When performing experiments, please use the consumables recommended in 2.3. When loading the consumables, please distribute the tubes evenly.

The following table lists different situations of loading consumables:



3.4 System Startup

Turn on the instrument, the POW is steady green, STU is off during self-check and hot lid preheating. When self-check and hot lid preheating is completed, the STU will turn to blue and instrument is ready for operation.



CHAPTER 4 SOFTWARE INTRODUCTION

4.1 Software Startup

After the instrument is turned on, the screen enters the self-test interface, as shown in the figure below. The instrument will perform self-test on the communication module, motion module, detection module and temperature control module in sequence.



After the self-test is successful, the operation screen enters the experimental interface.

4.2 Interface Introduction

The interface of the LTrtPCR1-X4/5 instrument is mainly composed of: main menu, submenu, and interface content.

	Sample		Sample Protocol Display						/sis
t	Sample Type	Empty	Sample No	Please enter	C/ul Please	enter CH	FAM CY	s vic	ROX
a	All	1	2	3	4	5	6	7	8
) t	A	Unknown 1 F/C/V	Unknown 2 F/C/V	Unknown 3 F/C/V	Unknown 4 F/C/V	Unknown 5 F/C/V	Unknown 6 F/C/V	Unknown 7	Unknown 8
p	в	Unknown 9 F/C/V	Unknown 10 F/C/V	Unknown 11 F/C/V	Unknown 12 F/C/V	Unknown 13 F/C/V	Unknown 14 F/C/V	Unknown 15	Unknown 16

1.Main Menu includes: Test, Data, Set, and Help.

- Test: For new experiments and setting up experimental information, such as sample information, experimental procedures, real-time monitoring of experiments and analysis of experimental data;
- Data: Used to record, query experimental data, and export experimental data and pictures;...
- Set: Used for instrument and user settings, currently including device information settings, language settings, and developer settings;
- Help: Indicate the installation of the PCR tubes.

NOTE:

Before starting the experiment, please confirm whether the temperature of the thermal lid in the settings-developer-thermal lid setting interface is correct, The default temperature of the hot lid is 110°C. If the temperature of the hot lid is incorrect, please go to ->Heat Lid Setting interface to set the temperature of the hot lid and save it (the temperature

data of the hot lid can only be saved successfully when the software communicates with the instrument normally). Start the experiment again. Otherwise, the experimental data may be abnormal.

4.2.1 Experiment Setting

4.2.1.1 Setting

Sample: To set the Sample Type, Sample No, Concentration of standards, Well position and Fluorescence Channel.

Click<Sample Type> a drop down box will appear, as shown in the figure below, you can select the corresponding sample type:

9		Sample			Protocol		Displa	y	Analy	sis
est	Sample Type	Empty	- Sa	mple No	Please enter	C/ul Please	enter CH	FAM CY	5 VIC	ROX
ata	All	Empty Unknown	2		3	4	5	6	7	8
et	A	Standard Positive Negative	kno 2		Unknown 3	Unknown 4	Unknown 5	Unknown 6	Unknown 7	Unknowr 8
elp	в	Unknown 9	Unkno 10	own	Unknown 11	Unknown 12	Unknown 13	Unknown 14	Unknown 15	Unknown 16

Click <Sample No> or <C/ μ L> to enter the sample ID or sample system, as shown in the figure below:

V1.0.1	0.1									202	23-01-13 13:17:55
0		Sample		Protocol			Displa	y		Analy	sis
Test	Sample Type	Empty 1	Sample No	Please enter	C/ul Ple	ase enter	сн	FAM	CY5	VIC	ROX
Data	AII	1	2	3	4		5	6		7	8
0	A	Unknown 12	Unknown 2	Unknown 3	Unknov 4	vn Unk	known 5	Unknow 6	n U	Inknown 7	Unknown 8
q	W	e	r	t s	у 6	u	7	8 İ	0	р	0
а	S	d	f	g	h		j	k		1	0
*	z	х	С	v	b	n		m	!	?	*
?123	,	•			Eng	lish					٢

You can directly click to select the desired optical path channel, as shown in the figure below:



Well Selection: You can click A /B/All to select A row , B row and All wells. Or click any well to select it.

9		Sample		Protocol		Display	′	Analysis		
st	Sample Type	Empty	Sample No	Please enter	C/ul Please	enter CH	FAM CY	5 VIC	ROX	
a	All	1	2	з	4	5	6	7	8	
t	A	Unknown 1 F/C/V	Unknown 2 F/C/V	Unknown 3 F/C/V	Unknown 4 F/C/V	Unknown 5 F/C/V	Unknown 6 F/C/V	Unknown 7	Unknown 8	
p	в	Unknown 9 F/C/V	Unknown 10 F/C/V	Unknown 11 F/C/V	Unknown 12 F/C/V	Unknown 13 F/C/V	Unknown 14 F/C/V	Unknown 15	Unknown 16	

Protocol: You can select and add the entire protocol of the experiment, as shown in the figure below, including the settings of temperature, time, Goto and cycle, and open the required protocol to carry out the corresponding experiment;

-			D			
2	Sa	mple	Protocol	Disp	Analysis	
est	Step	Temp Set	Hold time(S)	Goto	Cycle	Detection
0	1	95.0	60	0	1	
ata	2	95.0	15	0	1	
0	3	60.0	30	0	1	
Set	4	72.0	15	2	40	
2						
eip	₽Sa	ve 🖻 O	nen		bbA (+)	面 Del

NOTE: In the Detection column, signal collection must be turned on in the collection step.

Signal collection function is on ; Signal collection function is off. After complete the setting of Sample and Protocol, before Run the test, please confirm the hot lid setting is correct.

Click Run to start the test. The UI will pop up a reminder to set the File name, please set the File name or keep the default file name to continue the test. To terminate the test, please



NOTE: During the setting protocol of the LTrtPCR1 instrument, pay attention to setting the number of cycles in the last step of the amplification step process (as shown in the figure above, set to 40 cycles), and set the jump step in the jump bar, that is, from the jump step (the figure above is the 2nd Step) to the step of setting the amplification cycle (step 4 in the figure above) is the amplification step. For the remaining steps in the process, the cycle number can be set to 1, and the jump step can be set to 0. The steps that need to be detected need to select the detection switch.

LTrtPCR1 protocol setting:

V1.0.11.4	4				2023-05 16:54	
3	Sample	e Pro	otocol	Display	Analysis	
est	Step	Temp Set	Hold time(S)	Detect interval(s)	Detection	
9	Ť	60.0	1800	30		
ata						
•						
et						
?						
elp						
	🖹 Save	🗂 Open		€Ad	d 🗇 Del	

The constant temperature equipment needs to set the temperature, total time and detection interval, and pay attention to strobe the detection switch while setting the detection interval step.

Click Run to start the test. The UI will pop up a reminder to set the File name, please set the File name or keep the default file name to continue the test. To terminate the

test,please click Stop

C

Display: Real time display the running status and amplification curve, as shown in the figure below:

V1.0.10.1													2023-	01-13 11:28	
٢		S	ample			Protoc	ol			Dis	play		Analysis		
Test			All		FAM	CY5	VI	С				All	A	в	
0		. r										1			
Data	ata	1.00										2			
0	zed D	0.80										3			
Sot	rmali	0.60										4			
Jei	No	0.40										5			
?		0.20										6			
Help		0.00										7			
0		0	4	8	12	16 20 Cycle	24	28	32	36	40	8			
Stop		т	emp:72.0°C	1	Data	ont:7/41		Ren	naining 1	'ime:50:2	6				

Analysis: The experimental data can be analyzed, as shown in the figure below, which has the same function as the data-analysis. This page is mainly used to view and analyze the experimental data, and can view or export the experimental data of a certain channel or multiple channels.

S	ample		Pro	otocol		Display		А	nalysis	1
	All	FAM	CY5	VIC	ROX	All	Num	Туре	СТ	Copie
en 19					-	A7_VIC	7	Unknown	21.27	NAN
d Dai						A8_VIC	8	Unknown	22.31	NAN
alize						B1_VIC	9	Unknown	21.82	NAN
Lo						B2_VIC	10	Unknown	22.05	NAN
Z 020						B3_VIC	11	Unknown	22.57	NAN
0.10					CH3 0.00	B4_VIC	12	Unknown	22.54	NAN
0.00	4 8	12 16	20 24	28 32	36 40 44	B5_VIC	13	Unknown	21.53	NAN
		(Cycle			B6_VIC	14	Unknown	17.46	NAN
	Sa 000 010 010 010 010 010 010 010 010 01	Sample All 0.50 0.30 0.30 0.30 0.30 0.30 0.30 0.30	All FAM	Sample Pro All FAM CY5 050 030 030 030 030 030 030 030 020 030 030 020 030 030 020 030 04 8 12 16 20 24 Cycle 0	Sample Protocol All FAM CY5 VIC 000 0.00	Sample Protocol All FAM CY5 VIC ROX 0.00	Sample Protocol Display All FAM CY5 VIC ROX All All A7_VIC All A7_VIC All A7_VIC A8_VIC B1_VIC B2_VIC B3_VIC B4_VIC B4_VIC B4_VIC B4_VIC B5_VIC B5_VIC	Sample Protocol Display All FAM CY5 VIC ROX All Num 030 <td< td=""><td>Sample Protocol Display All Num Type All FAM CY5 VIC ROX All Num Type 0.00 0</td><td>Sample Protocol Display Analysis All FAM CY5 VIC ROX All Num Type CT 0.00 0</td></td<>	Sample Protocol Display All Num Type All FAM CY5 VIC ROX All Num Type 0.00 0	Sample Protocol Display Analysis All FAM CY5 VIC ROX All Num Type CT 0.00 0

4.2.2 Data

The data interface is shown in the figure below, which is used to query data.

V1.0.1	0.1				2023-01-13 13:18:46
() Test	Start Date 2023-01-13	End Date 2023-01-13	Q	1 2 File name select	Q
Q Data	Number	Test time		Test Name	
0 Set					
? Help					
Run	Status: ready				

V1.0.1	J.1		nQ16T-X4	8		2023-01-13 13:19:02
G	Start Date	End Date			1= 2= Import File	
Test	2023-01	⁻ hu, Jan 12, 2023				ne select
Data	Number	Dec	11	2022		st Name
0		Jan	12	2023		
Set		Feb	13			
? Help		Cancel		_	ок	
Run		dy				

Select Start Date and End date to search the historical data.

Import File: To open the historical test file stored in local or USB disk. If the data stored in

USB disk, insert the USB Disk, then click to import the historical test data.

< nQ1		
//sdcard/nQ1	6/data/	
111 1项		
202 2 项	2-12-29-16-31-29	

NOTE: If you need to export data, please insert the U disk in advance.

4.2.3 Set

The setting interface is shown in the figure below, which is used to set the relevant information of the instrument.



Device Information : You can find the basic information of the instrument.

V1.0.10.1		2023-01-13 13:22:10
J Test Q Data Set	Product nameSoftWare versionV1.0.10.1PCB version1006221114000020Hardware versionAlpha.2212050020SNINT22601000101Optical channels4	
Help	Back	J
Run	Status: ready	

Language settings: You can choose two languages: Chinese and English, as shown in the figure below.



Developer settings: If you need to use the developer mode, please contact the supplier or

dealer.

4.2.4 Help

To indicate the correct placement of PCR tube in the heating block.



CHAPTER 5 APPLICATIONS

5.1 HBV RT-PCR Assay

TaqMan probe method requires a sequence specific, fluorescent labeled oligonucleotide probe and a pair of specific primers. During the amplification reaction, the probe is hybridized with the target, the 5'-3' exonuclease activity of DNA polymerase removes the reporter group, the reporter group is separated from the quenching group, and the fluorescent signal generated is proportional to the amplification product in the sample.

HBV belongs to the hepatotropic DNA virus family, with a gene length of about 3.2kb. It is part of double stranded circular DNA. By using specific primers and fluorescent probes designed for the conserved region of DNA, real-time fluorescent quantitative PCR technology is applied to realize the quantitative determination of HBV DNA by monitoring the changes of fluorescent signals.

5.1.1 Preparation of qPCR Reaction Solution

PCR total reaction volume (25µL):

Reagent	Volume (µL)
PCR reaction solution (including internal standard)	15
Enzyme mixture	2
Specimen to be tested	8

The experiment program as below:

	Progra	am	
1	25 ℃	3min	
2	95 ℃	5min	
2	95 ℃	15s	40oveloo
3	60 ℃ (End point)	30s	40Cycles
4	40 ℃	10s	

5.1.2 Instrument Startup

Turn on the instrument. After instrument self-check and hot lid preheating is finished, the status light will turn to blue light and it is ready for operation.

5.1.3 New Experiment

Click<Test>-Sample to select the well position, sample type, enter sample No and select the Fluorescence channel. If the sample is standard, please enter the concentration at C/ul.

3		Sample		Protocol		Displa	у	Analy	Analysis		
est	Sample Type	Empty	Sample	Please enter	C/ul Please	enter CH	FAM CY	5 VIC	ROX		
ata	All	1	2	3	4	5	6	7	8		
D et	A	Unknown 1 F/C/V	Unknown 2 F/C/V	Unknown 3 F/C/V	Unknown 4 F/C/V	Unknown 5 F/C/V	Unknown 6 F/C/V	Unknown 7	Unknowr 8		
elp	в	Unknown 9 F/C/V	Unknown 10 F/C/V	Unknown 11 F/C/V	Unknown 12 F/C/V	Unknown 13 F/C/V	Unknown 14 F/C/V	Unknown 15	Unknowr 16		

5.1.4 Protocol Setting

Click Add to add steps and edit the temperature, hold time, cycle and signal detection.

V1.0.11.	4					2023-05-0 17:20:4	
3	Sa	mple	Protocol	Display		Analysis	
Test	Step	Temp Set	Hold time(S)	Goto	Cycle	Detection	
0	1	95.0	60	0	1		
Data	2	95.0	15	0	1		
0	3	60.0	30	0	1		
Set	4	72.0	15	2	40		
	🖹 Sa	ve 🗂 O	pen		⊕Add	Del	
Run	Stat	us: ready					

5.1.5 Load the Reaction Tube

Click the door button to open the lid cover, load the reaction tube.

5.1.6 Run the Experiment

Please confirm the hot lid temperature before run the experiment.

5.1.7 Experiment Complete

After the experiment is completed, a Note message will pop up to remind the test completed.Click OK to close the window.

٥		Sample	Protocol	Display	play		Analysis		
Test		All	· · · ·		Num	Туре	ст	Copies*	
0	g	3000	Note		12	Unknown	NA	NA	
Data	d Dat	2900		и			NA	NA	
~	alize	2800		, v			NA	NA	
0	lorm	2700	Test completed	м	4		NA	- NA	
Set	Z	2500		, vi			NA	NA	
0		2400		ų			NA	NA	
		0.0	ок	y.			NA	NA	
Help			Cycle	AS_FAM					
0	Tł	nreshold	Stid Curve	Export Pics E	cport Data		Save Da	ta	
Run		Status: ready							

5.1.8 Amplification Curve

Amplification curve and standard curve as shown below, the CT value and concentration is displayed.

9		Sample			Protocol		Display		Analysis				
Fest			All	FAM	CY5	VIC	ROX		All	Num	Туре	CT	Copies
0	ta a	50							A7_VIC	7	Unknown	21.27	NAN
Data	dDa	40							A8_VIC	8	Unknown	22.31	NAN
	alize	20						- 1	B1_VIC	9	Unknown	21.82	NAN
•	lorm	30						- 1	B2_VIC	10	Unknown	22.05	NAN
Set	Z 0.	20						- 1	B3_VIC	11	Unknown	22.57	NAN
0	0.	10					CH3	0.00	B4_VIC	12	Unknown	22.54	NAN
	0.	00	4 8	12 16	20 24	28 32	36 40	44	B5_VIC	13	Unknown	21,53	NAN
Help				(Cycle				B6_VIC	14	Unknown	17.46	NAN
	Theo	abold				1 Cumus	5	Dies	Euro	ert Dete		0.	

CHAPTER 6 MAINTENANCE

6.1 Contamination Cleaning

In order to ensure the performance and lifetime of instrument, and also reduce contamination, the instruments needs to be cleaned regularly every week.

1. If contamination occur, use clean rag with 70% ethanol to wipe all the accessible parts (heating block, hot lid, sealing ring etc), after the ethanol is completely evaporated, use a new rag with pure water to wipe the parts again, then wait it to dry naturally.

2. If dirt or liquid dropped into well of heating block, please use a cotton swab with 70% ethanol to wipe the well, after the ethanol is completely evaporated, use a new swap with pure water to wipe the parts again, then wait it to dry naturally.







Ethanol/water

Clean rag

Clean swap



Clean the hot lid

Clean the heating block

6.2 Fan and Vent Cleaning

According to the using frequency of the instrument, fans and vent net need to be cleaned regularly for half a year. The fan is located on the instrument backplane as shown below, and the vent net is under the instrument. Using an air blower to clean the dirt adhered on the vent net and fun.



6.3 Fuse Change

User a screw driver to unload the socket of fuse and take out the fuse, then replace a new one with same specification (3.15A).



6.4 Instrument Locked

The transportation lock is specially designed for the transportation of the instrument. Please lock the instrument if you need to ship the instrument. Refer to contents of 3.2.3 for locking the instrument.



WARNNING:

Power off the instrument before any cleaning, changing.

Don't drop any liquid or nut into instrument.

Do not spread water or ethanol to the parts, please use rag or swap wet with water or ethanol to clean it.

Do not power on the instrument before it dry naturally.





CHAPTER 7 TROUBLE SHOOTING

No.	Issue	Possible causes	Processing method
1	Power on the instrument,	The power cable is not connected	Plug the power cable again and fasten
	no response from	reliably.	it.
	instrument and the	The power supply voltage is not	Ensure the mains input voltage is
	power indicator is not	correct.	110V-220V and the power supply is
	light on.		properly grounded.
		Fuse is broken	Replace the fuse, refer to chapter 6
2	Power on instrument,	The connection cable of display	Contact after sales service
	screen has no response	screen is loose.	
		Screen is damaged	Contact after sales service
3	STU indicator flashes	Instrument is locked.	Please unlock the instrument, refer to
	quickly after power on.		3.2.3
		Instrument error	Contact after sales service
4	The instrument fails to	There is failure in heating module	Contact after sales service
	pass self-test.	or optical module.	
5	The block or hot lid does	The temperature fuse, heating	Contact the after sales service
	not heating or cooling.	element or temperature sensor is	
		damaged.	
6	After program ends, the	The vent is blocked	Clear the blockage of the vent
	instrument cool down at	Ambient temperature is	Keep the indoor temperature at 10-30 $^\circ C$
	a obvious slow speed	excessively high	
		The fan failed.	Replace the fan.
7	When do empty block	Check whether there is strong light	Avoid strong light.
	scanning or empty tube	inference the instrument.	
	scanning, the signal is	Check whether there is spilled	Please refer to chapter 6 to maintain the
	abnormal.	liquid or dirt in the well.	well
8	No signal data collected	Check the program setting	End point must be set in the
	when conduct the empty		fluorescence acquisition step
	background scanning or	Check whether the communication	Restart the instrument and software,
	sample scanning	between the instrument and	reconnect and test again.
	verification	software is in good condition.	
	Denti	The detecting module failed.	
9	Reagent is evaporated	incompatible PCR tube or PCR	It is recommended to use
		tube is defective and not tightly	vendor-recommended consumables.
		closed; incorrect use of the tube	
		cap.	