Real-Time PCR Detection System

User Manual

(For X960A/X960B)

Heal Force

Heal Force

Contents

Section V - Test Introduction and Description

Section VI - Other Function Introduction

Introduction

How to use this manual

About this manual

This manual is intended to solve your any question and technical problem in installation, operation and maintenance of this equipment, and provides instructions on proper installation, use and maintenance of this instrument.

Safety Cautions

Before installation, use and maintenance of this instrument, read and observe any cautions and considerations in this guide to ensure normal operation of the equipment and user's personal and property safety.

Instructions on Safety and Use

Considerations for Use

Operation Safety

- The power supply for this instrument must be earthed reliably. A three-pole plug is provided for this instrument, with the additional pole connected to earthing wire, which shall be used with an earthed power socket. Before connecting power supply, ensure it meets the voltage requirement for this instrument, and make sure rated load of the power socket is not lower than the load requirement for this instrument.
- In case of any damage to the power cable, replace it with a new one of the same model and specification. No object shall be placed onto the power cable. The cable shall not be located at area subject to busy traffic of persons. Connect/disconnect the power cable by holding the plug rather than any portion of the cable itself.
- As high heat will be generated during operation of the instrument, do not touch any metal module of the instrument with any part of body to prevent burn.
- No object shall be located within an area of 30cm around the instrument to ensure good heat dissipation during operation.

Maintenance Safety

- Modules of this instrument shall be maintained to ensure precision of testis is recommended to clean the instrument with clean soft cloth soaked with absolute alcohol. Do not use any corrosive detergent or make any detergent

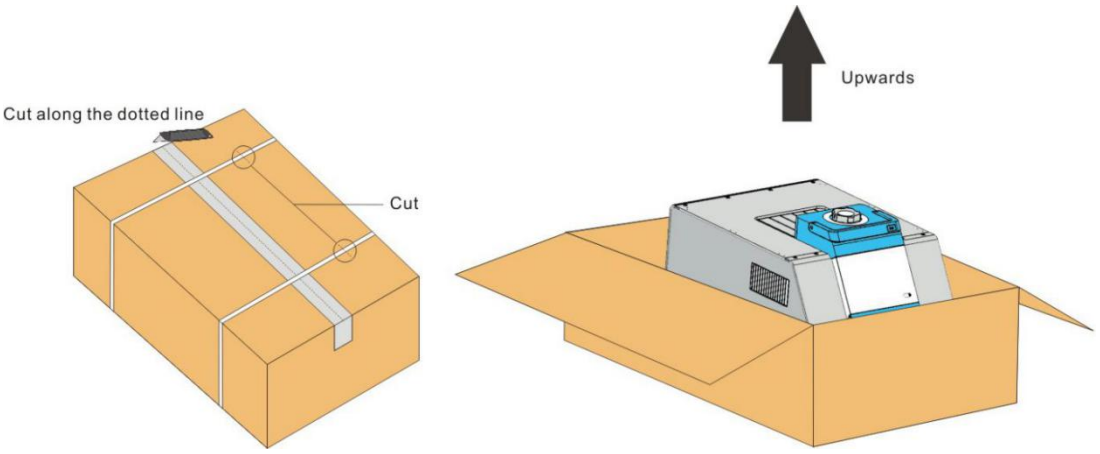
enter the instrument to prevent any damage to it.

- Immediately switch off the power supply, stop test and contact your supplier or any professional service personnel in any of the following cases:
  1) Any reagent, water or other liquid enters the instrument.
  2) The instrument falls down from an elevation or the enclosure is damaged.
  3) The instrument generates any abnormal noise or pungent smell during operation.
  4) Functions of the instrument vary substantially, such as failure to start/shut down or operate it normally.

Section I - Instrument Installation

Locating the Instrument

- This instrument must be located properly after being unpacked.
- Place the packing case upright in horizontal position, cut off the packing strip around the case and the adhesive tape sealing the case.
- Take out the accessories of the case and store them in a secure place for future use.
- Remove EPE foam covered on the instrument, and lift it up by supporting its bottom with two persons, and then locate it on a proper lab desk. Handle the instrument with caution.
- Store the packing case properly for containing in future.

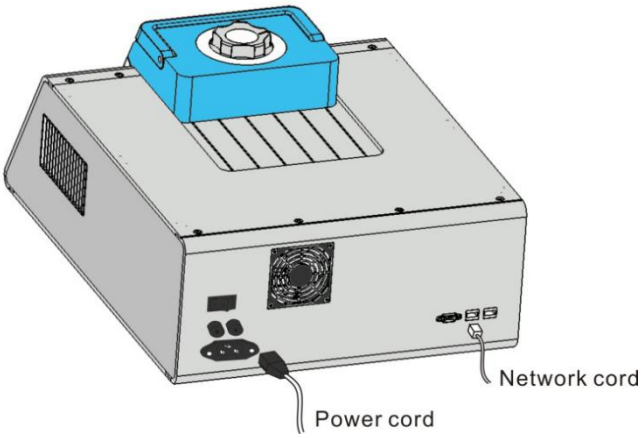


Risk of personal injury! Unless you have been trained properly, do not attempt to lift the instrument or any heavy component of it. Lifting the packing case in any improper way may cause ache in or permanent injury to body, or fall of the instrument. Lift or move the instrument with proper handling tool or in a correct way. Two persons must be used to lift the instrument up.

This instrument shall be mounted upright on a horizontal surface, around which there shall not be any vibration, EMI or any high-inductance equipment (e.g. refrigerator, centrifuge, and stirrer).

Installation of Instrument

Place the instrument on a testing platform and then take out the power cable provided along with it. Connect the power cable by inserting the plug at one end of the cable into the socket at the rear of the instrument, and inserting the plug at the other end into a power socket earthed properly.

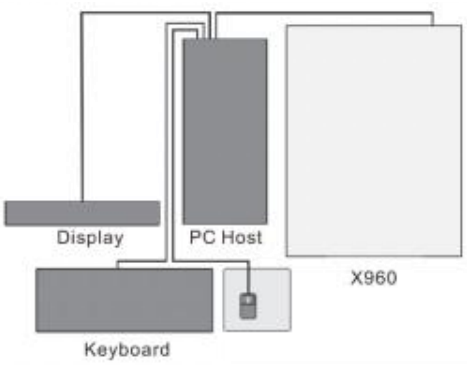


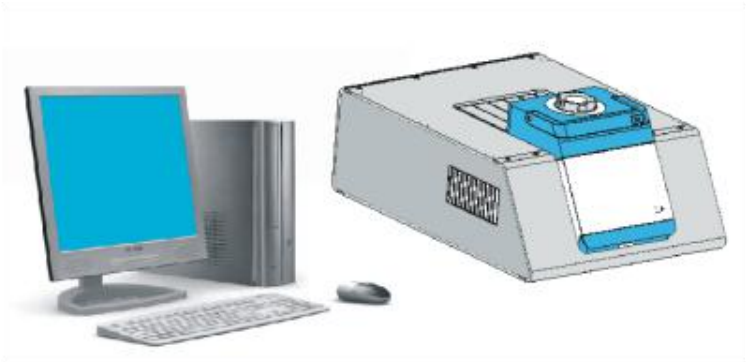
Danger Do not touch the power cable with wet hand. Do not disconnect the cable during operation of the instrument. In case of any damage in the power cable, replace it with an acceptable one. The instrument shall be always connected to a socket earthed properly. When the power supply is disconnected, connect one end of the network cable provided to the LAN port at the back of the instrument and the other end to the LAN port on a PC.

Danger Do not connect power supply until the instrument has been connected to a PC network cable.

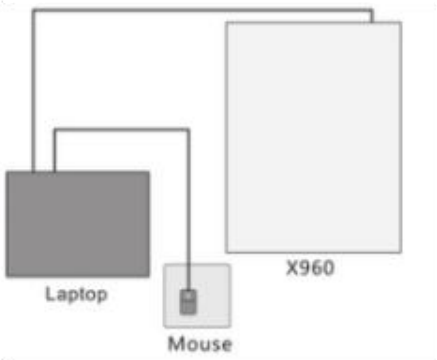
Connection of Instrument

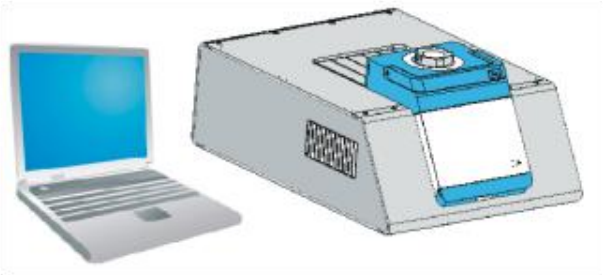
Connect PC to the instrument





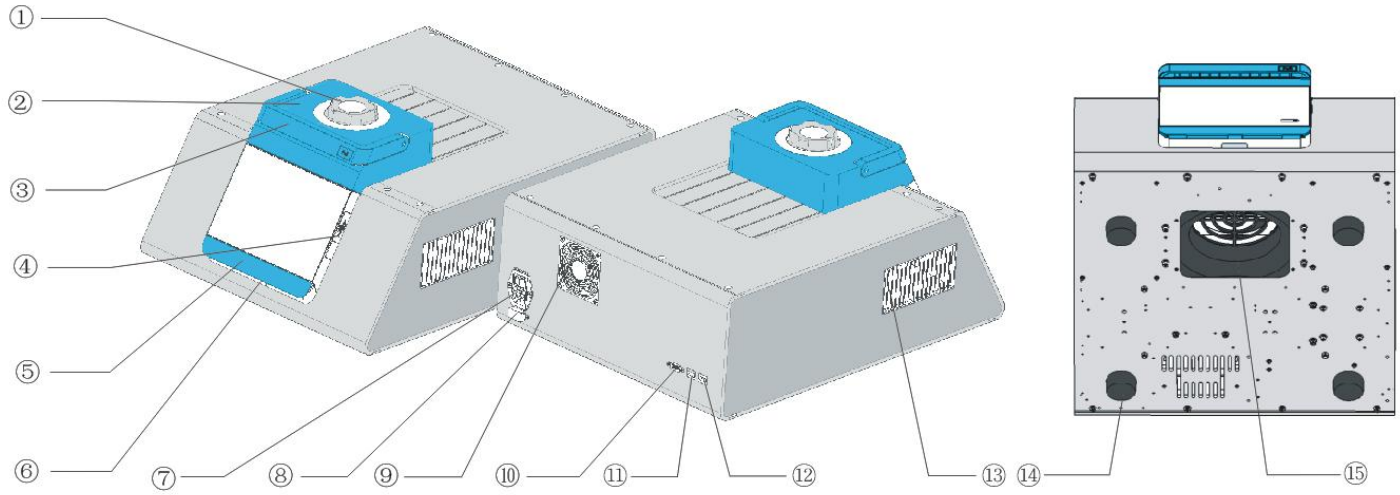
Connect laptop to the instrument





Section II - Instrument Appearance and Internal Components

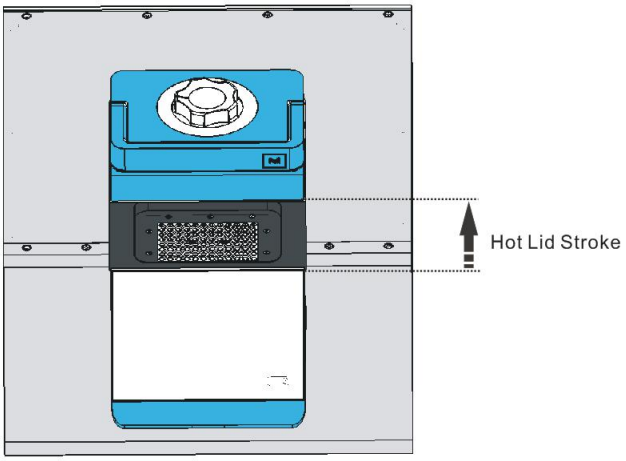
Overview



① Knob ② Lid ③ Handle ④ USB Port ⑤ Pad Tray ⑥ Operation Indicator LED
⑦ Power Switch ⑧ Power Socket & Fuse ⑨ Air Outlet ⑩ 232 Interface ⑪ LAN Port
⑫ WAN Port ⑬ Air Outlet ⑭ Foot Pad ⑮ Air Inlet

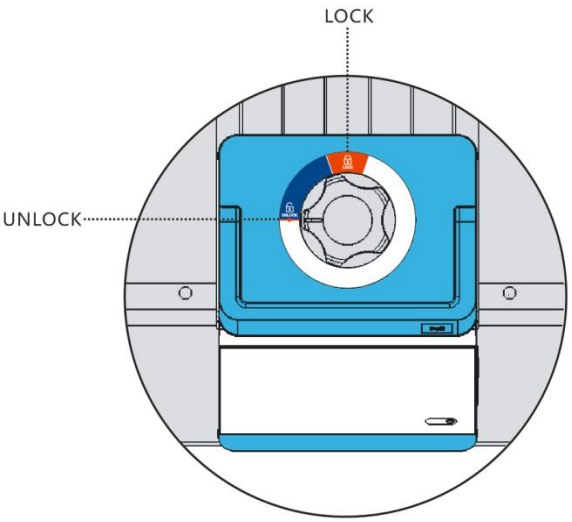
Construction & Function

The lid is operated with a sliding guide to a range as customized by the user. As shown in the figure at the right side, you switch the knob to the point UNLOCK and slide the lid backwards to load the test tube into the instrument, and then close the lid by sliding it forwards and switch the knob to a proper position to run the program for testing.



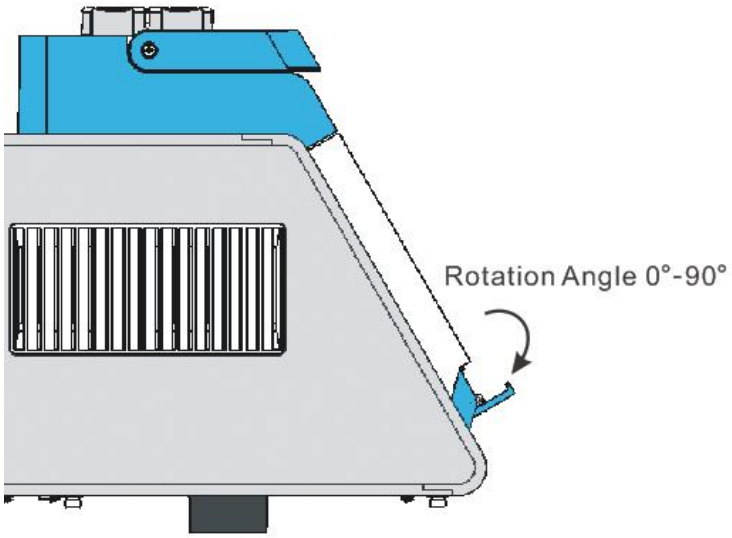
Warning The handle provided on the instrument is intended for push/pull. Do not lift up or press down the handler with force to prevent damage to the lid structure.

When the knob is switched to the point UNLOCK, the lid can be slide back and forth for loading/unloading of test tube. After loading the test tube, close the lid and rotate the knob clockwise to a proper position and run the program for testing. It is recommended to align mark on the knob with the point LOCK.



Warning Do not slide lid component forwards/backwards when the mark aligns with LOCK to prevent damage to the lid.

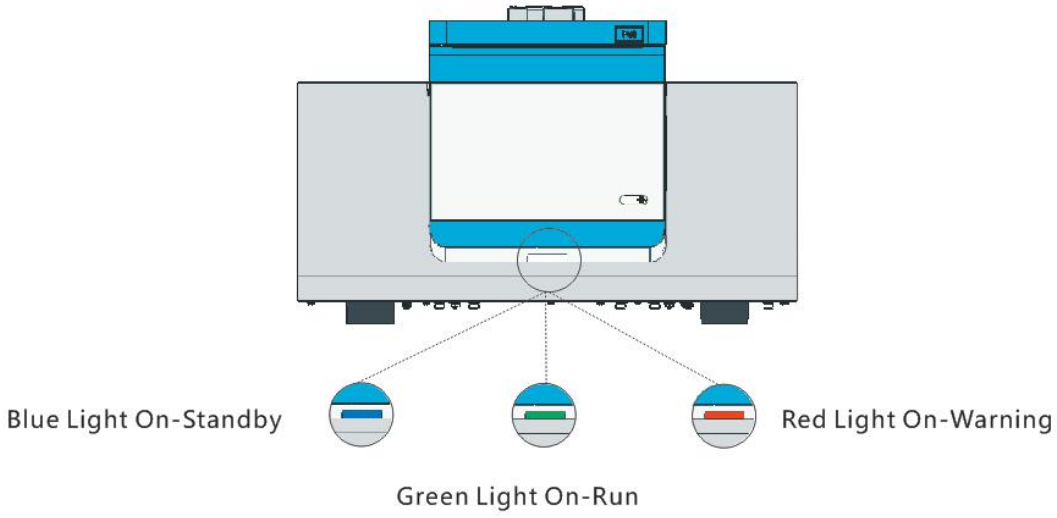
Turn pad tray down clockwise to a position at an angle of 90° to the enclosure to open the tray, and then place a pad on the tray.



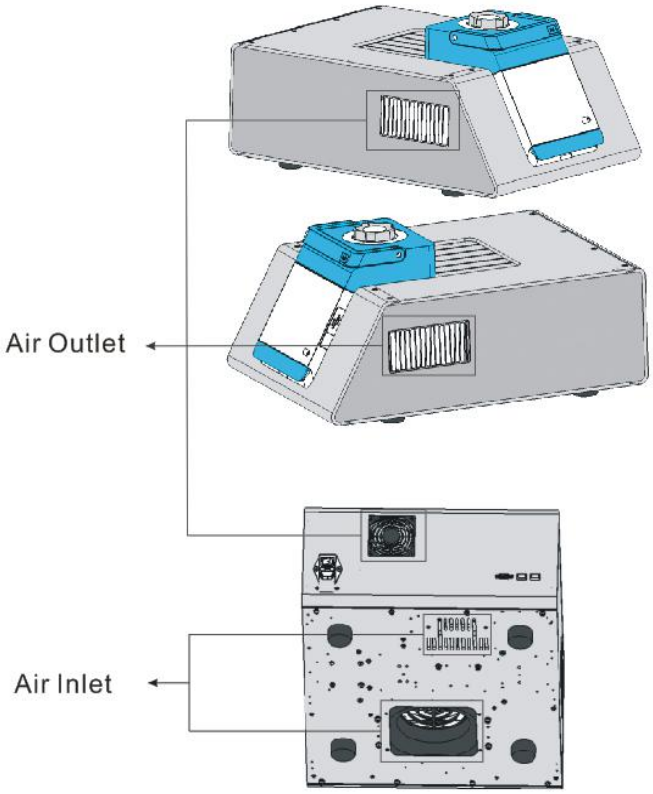
Warning

For this instrument, the tray moves in a range between 0°~90° to the enclosure; do not move the tray out of this range to prevent damage to the instrument.

There is a status indicator LED under the front panel, which turns on in any of three colors - blue, green, and red, respectively indicating Standby, Operating, and Alarm.



To ensure a safe and reliable testing procedure, place this instrument on a flat platform, and reserve areas of at least 30cm wide around the four sides of it, so as to make sure proper ventilation and heat dissipation.



Do not place any object (e.g. paper, plastic film) at the air inlet to prevent it from being absorbed in and thereby permanent damage to the fan or cooler and damage to the instrument resulted from overheating of module component due to poor ventilation.

Do not place any object at any of the air outlets to prevent overheating and thereby permanent damage to the instrument resulted from poor ventilation.

Do not place any inflammable, explosive or heat-sensitive material near any air outlet to prevent property damage or potential accident.

Section III - Instrument Description and Performance Properties

Instrument Description

[Product Name]

Real-time fluorescence quantitative PCR unit

[Product Model]

X960A

X960B

[Construction]

It consists of power module, control module, driver module, display module, heated lid module, sample base module, camera module, light source module, and optical fiber module.

[Application Range]

It is applicable to polymerase chain reaction (PCR).

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Instrument Performance Indicators

Product Name:	Real-Time Fluorescence Dosing PCR Unit	
Product Model:	X960A	X960B
Compatible OS:	Windows XP/VISTA/Windows 7	
Bits:	32/64	32/64
Minimum Memory:	512M	512M
Display Unit:	256MB	256MB
CPU:	Pentium IV 1.6GHZ or higher	
Overall Dimensions:	590mm (L)*442mm (W)*285mm (H)	
Weight:	28.5kg	28.7kg
Connection Pattern:	LAN WIFI	LAN WIFI
Power Supply:	AC 220V 50HZ/60HZ	
Input Power:	1000VA	1000VA
Fuse:	AC 250V 10A	AC 250V 10A
Sample Capacity:	96 holes x 0.2ml	96 holes x 0.2ml
Light Source:	Ultrahigh-luminance mono-color LED	Ultrahigh-luminance mono-color LED
Detector:	High-sensitivity refrigerator CCD	High-sensitivity refrigerator CCD
Dynamic Testing Range:	10^0-10^{10}	10^0-10^{10}
Minimum Testing Template:	Single copy	Single copy
Reaction Volume:	15μl-100μl	15μl-100μl
Fluorescence Excitation Wavelength:	470nm-525nm	470nm-625nm
Fluorescence Detection Wavelength:	520mm-570mm	520nm-675nm
Fluorescein and Dye Detected:	Channel 1:FAM, SYBR Channel 2:VIC, HEX, JOE, TET, TAMRA	Channel 1:FAM, SYBR Channel 2:VIC, HEX, JOE, TET, TAMRA Channel 3:ROX, TEXRAD Channel 4:CY5 Channel 5:Reserved for user
Temperature Range:	0℃~100℃	0℃~100℃

Heating Rate:	≥5℃/s (45℃—99℃)	≥5℃/s (45℃—99℃)
Cooling Rate:	≥5℃/s (45℃—99℃)	≥5℃/s (45℃—99℃)
Temperature Control Uniformity:	At constant 95 ℃ within 10s:±0.4℃ at constant 55 ℃ within	At constant 95 ℃ within 10s:±0.4℃ at constant 55 ℃ within
Temperature Accuracy:	≤±0.1℃ (45℃≤T≤95℃)	≤±0.1 ℃ (45 ℃≤T≤95 ℃)
Gradient Temperature Width:	1℃—30℃	1℃—30℃
Gradient Temperature Range:	30℃—100℃	30℃—100℃
Heated Lid Temperature Range:	(Rt +2℃) —110℃	(Rt +2℃) —110℃
Temperature Control Mode:	Semi-conductor thermoelectric module	Semi-conductor thermoelectric module
Temperature Control Programming:	Max. 999 cycles	Max. 999 cycles
Special Functions:	Threshold analysis, dissolution curve analysis, grade, and	

Operating Environment and Storage Condition

Operating Environment	**Ambient temperature**	10℃~30℃
	Relative humidity	< 70%
Storage Condition	**Ambient temperature**	-20℃—55℃
	Relative humidity	< 80%

To ensure Normal Operation of the instrument and accuracy of test result, be sure to operate/store the instrument in accordance with the specified operating/storage conditions; or otherwise the instrument may be damaged or personal injury may be caused to the operator.

Section IV - Use and Installation of Software

Installation Instructions

Preparations

Items to be prepared: instrument, PC, and connecting wires

Installation Environment

Note: A genuine edition of windows XP/VISTA/7/10 shall be used, or otherwise any compatibility error may occur. Shut down any anti-virus software running in the computer during installation to prevent any false alarm (which shall be ignored if occurring). Shut down any firewall installed in the computer.

Installation Considerations

Note: To avoid failure of installation resulted from any error occurring during installation and facilitate your installation, please observe the following procedure.

Before Start

Note: Before installation of the software, set your computer properly to prevent interruption of test and thereby data loss.

Note:Do not install on system disk.

Note:Cable and wireless networks can only choose one!

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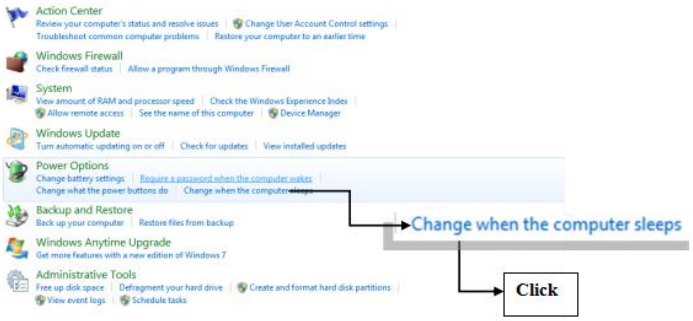
Computer Setting (Win 7)

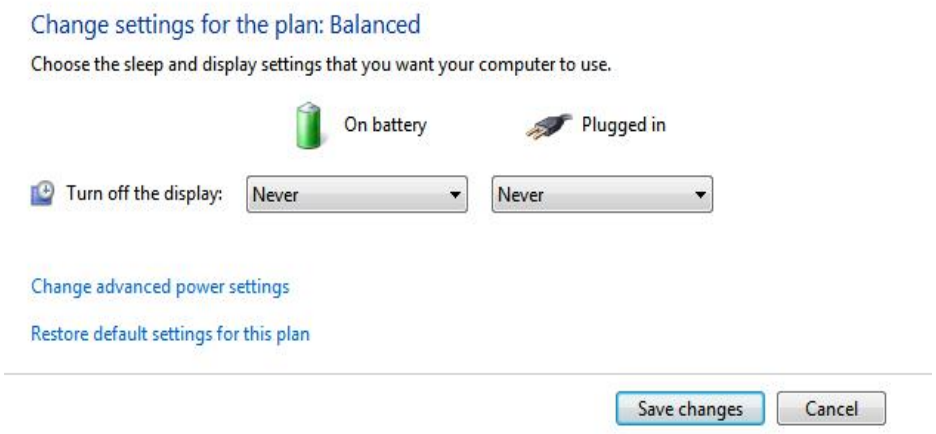
Display Setting:

1. Navigate  to Start > Control Panel.

2. Select "Power Options" in Control Panel.

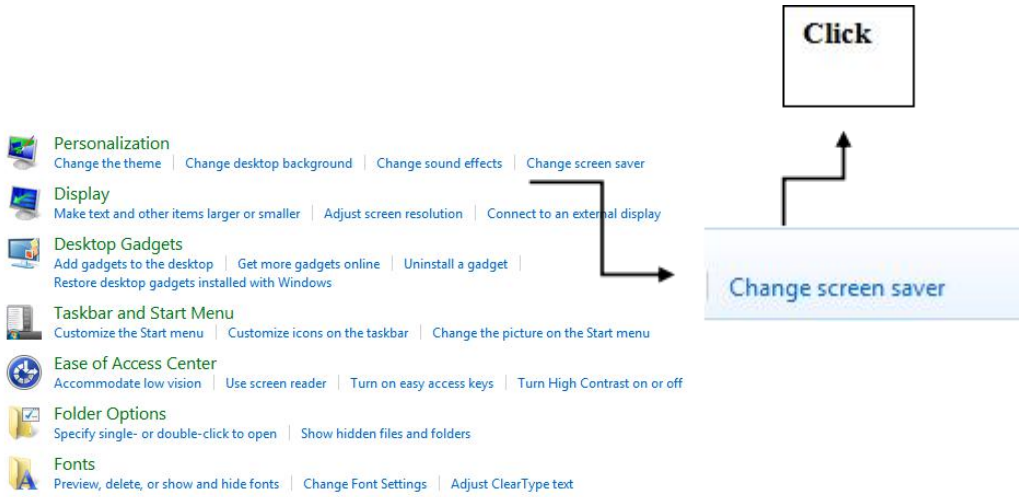


3. Select "Change when the computer sleeps" in the window

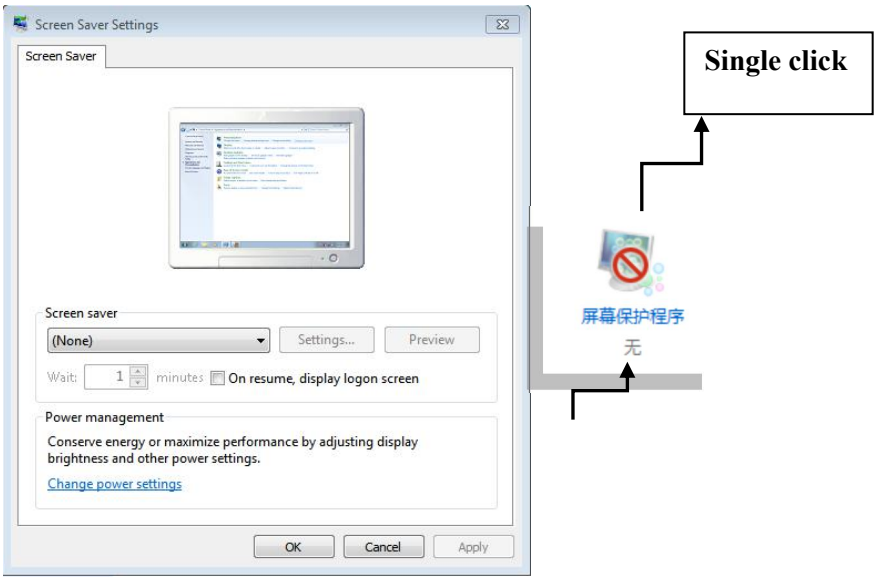


4. Select "Never" under "Turn off the display" in the window
   Select "Never" under "Put the computer to sleep".

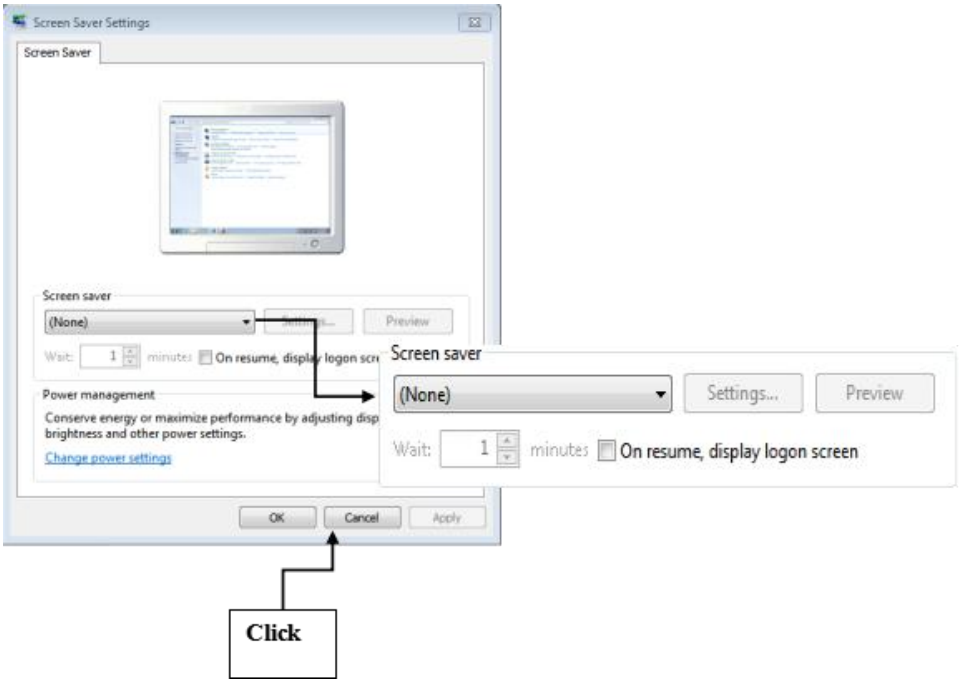
5. Return to the window in Step 3 and click on "Personalization" button.



6. Click on "Screen Saver" at the lower right corner.



7. Select "None" from the drop down list in the dialog box.



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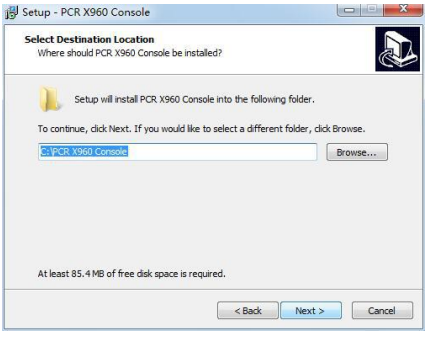
Start Installation

Step 1: Access the setup package from the flash disk provided.

Step 2: Double click on the setup file and choose a proper installation language.

Step 3: Read the pre-installation instructions.



Step 4: Choose a destination folder to install the program.

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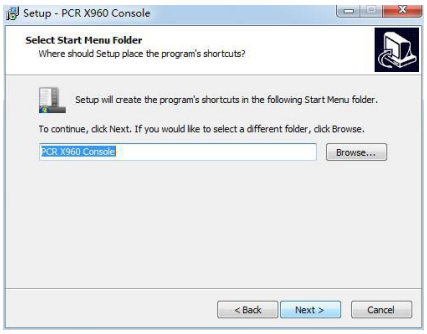
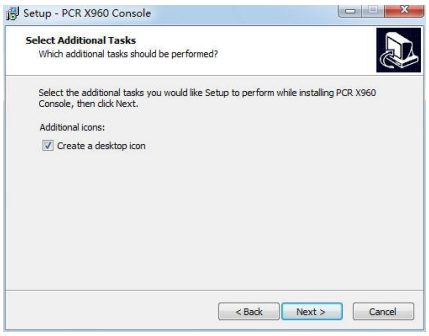
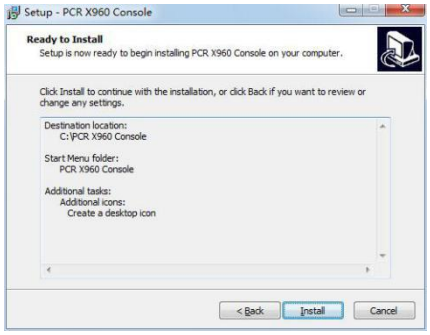


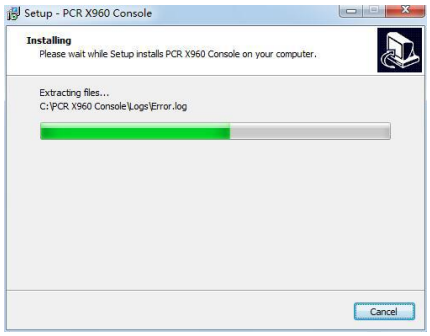
Step 5: Choose name of the folder to be created under start menu.



Step 6: Choose whether to create a shortcut on desktop.

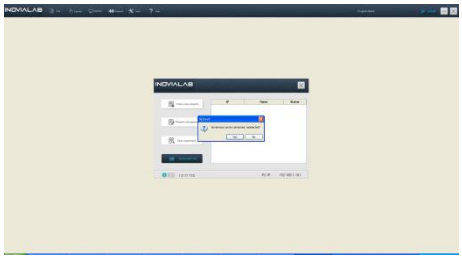


Step 7: Confirm any information before installation



Step 8: Install the program.

Step 9: Finish the setup wizard, and check whether RealGen is running; check the option and click on Finish.



Step 10: Open page of RealGen.

Step 11: Click on Yes for automatic connection.

Step 12: If it is indicated that there is no available computer to connect, check whether the computer has been started and the network is operated properly, or the instruction has been connected to any other computer.

Notice

If the instrument connection fails, check whether it has been properly connected to the computer; if yes, shut down any anti-virus software and restart the instrument, and then try connection after it beeps. If connection fails again, contact the dealer for a solution.

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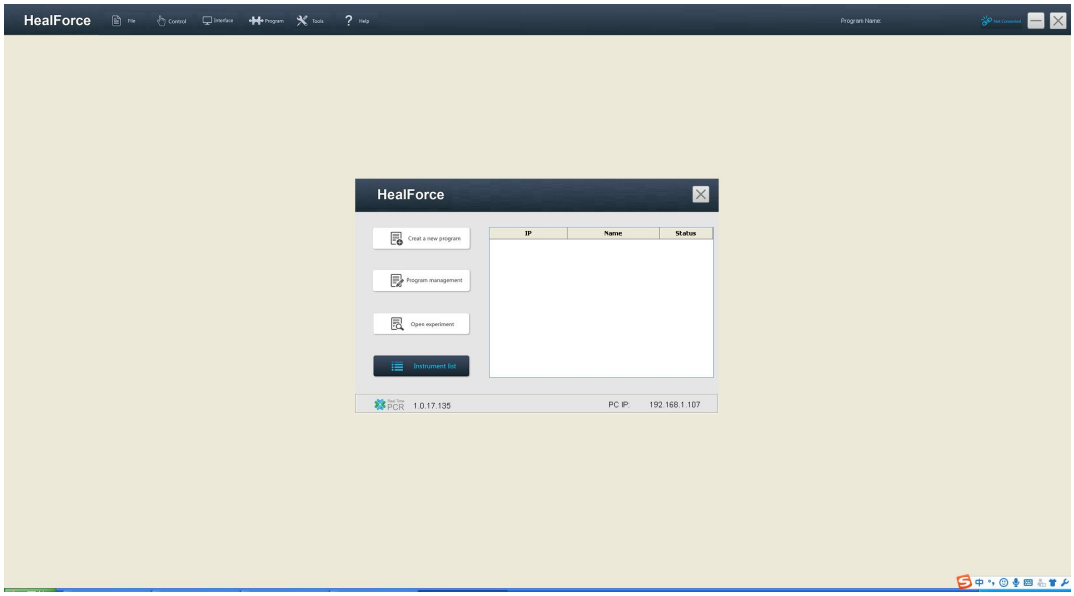
Overview of User Interface

Interface Overview



Toolbar - File:

Load Test: Load a finished test file for display and analysis.

Export Test: Export a finished test for data storage.

Load Project: Load a finished project file to start a test.

Export Project: Export a loaded project for data storage.

Import Standard Curve: Import a standard curve with similar circumstance for analysis.

Running Logs: Display any projects running currently in the program.

Print: Print desired information.

Patient Information: Import patient information for printing and data analysis.

Toolbar - Control:

Disconnect: Disconnect the program from the instrument

Connect: Manually connect the program to the instrument by entering its IP address (the default is "192.168.1.6”").

Pause: Pause operation of the project, which will be resumed in 5 minutes if no operation has been performed.

Stop: Cease operation of the project.

Operate:

1. Resume a project which is paused, or
2. Operate a chosen project when the instrument is idle.

Toolbar - Interface:

Monitor: Switch to real-time monitoring page.

Analyze: Switch to subsequent analysis page (note: this makes sense only after a test file has been loaded).

Toolbar - Project:

Change parameters: After a test has been loaded, when current settings are not proper to the expectation, they may be changed under this option. Any change will be applied immediately after completion. Change to parameters makes sense only after a test has been loaded.

Project Wizard: Call a project wizard dialog box

Toolbar - Utilities:

TM calculator: Call a TM calculator

Gradient calculator: Call a gradient calculator.

Chinese-English toggle: Toggle between Chinese and English language.

Toolbar - Help:

Instructions: Open instructions in the program

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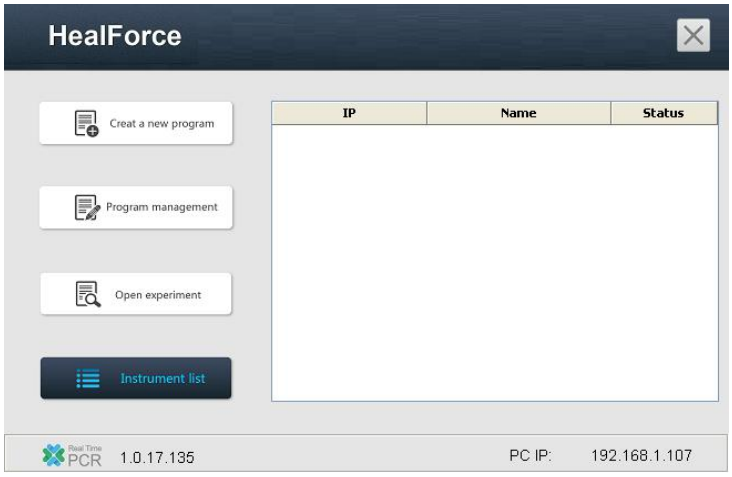
Project Wizard Interface:



New Project: Create a new project for test.

Project Management: Open a project list and manage any project in it, and operate or modify a project.

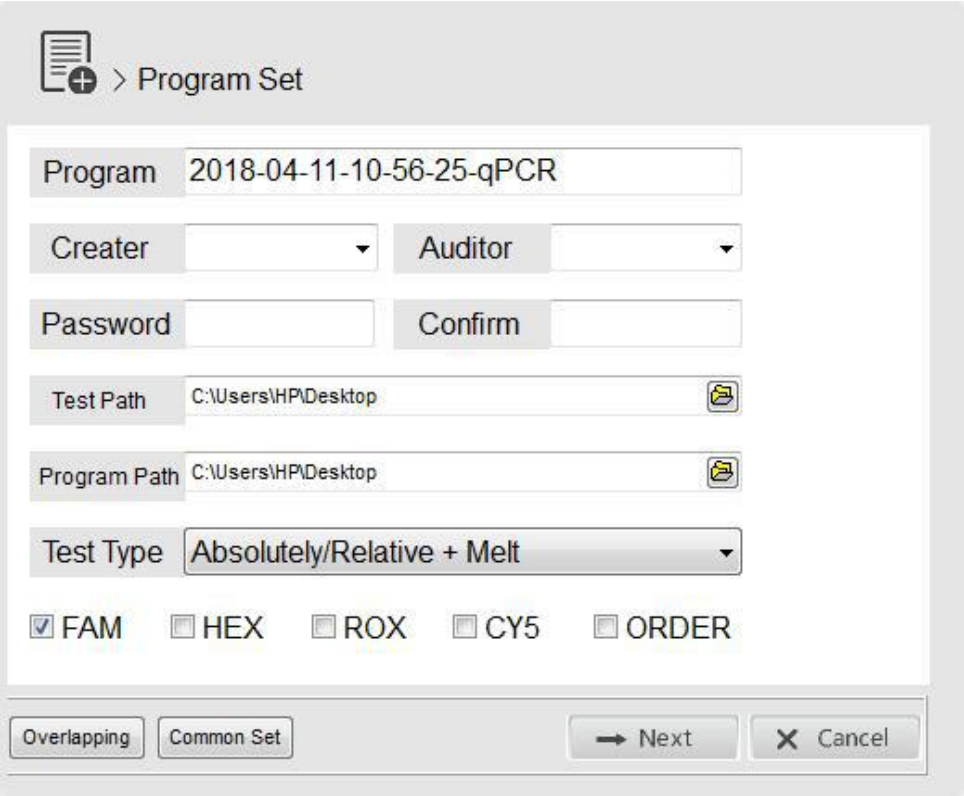
Open Test File: Open a test file list and manage any test file, and load or delete a file.

Instrument List: Open an instrument list, and connect or rename any instrument in it.

Software Version: Display current software version number.

Current Address: Display IP address of the computer.

New Project = Basic Settings



Project Name: Name the project to be saved.

Creator: Specify a creator for the project, and save it by pressing ENTER. This option can be left blank.

Reviewer: Specify a reviewer for the project, and save it by pressing ENTER. This option can be left blank.

Password: Specify a password to access this project. It can be left blank.

Confirm the password: Confirm the password by repeating it.

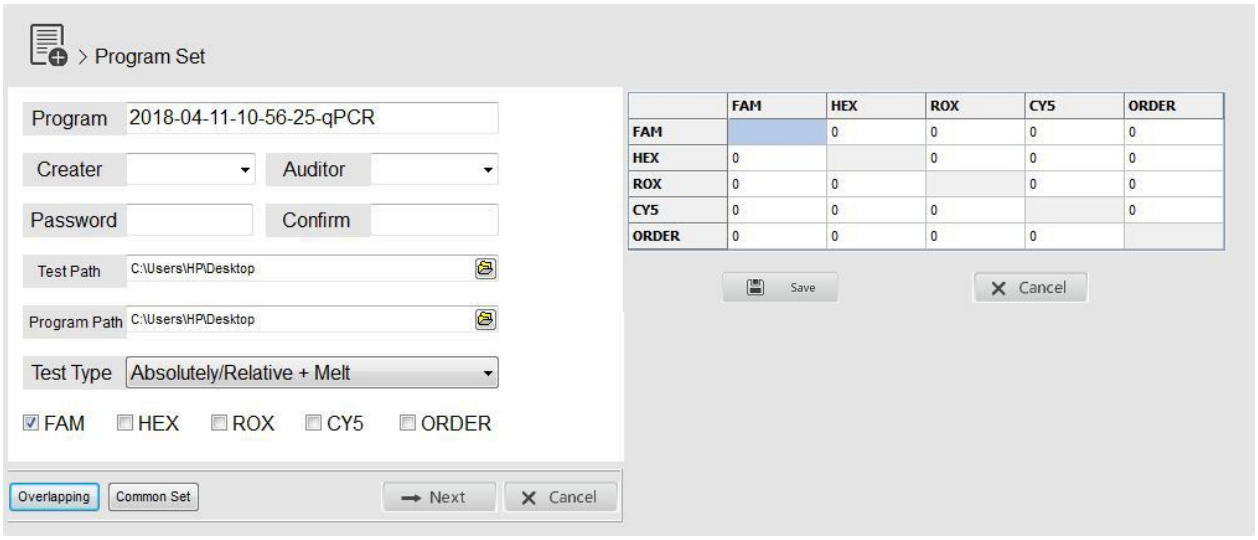
Test Result Path: The path where the test result is saved.

Project File Path: The path where the project file is saved.

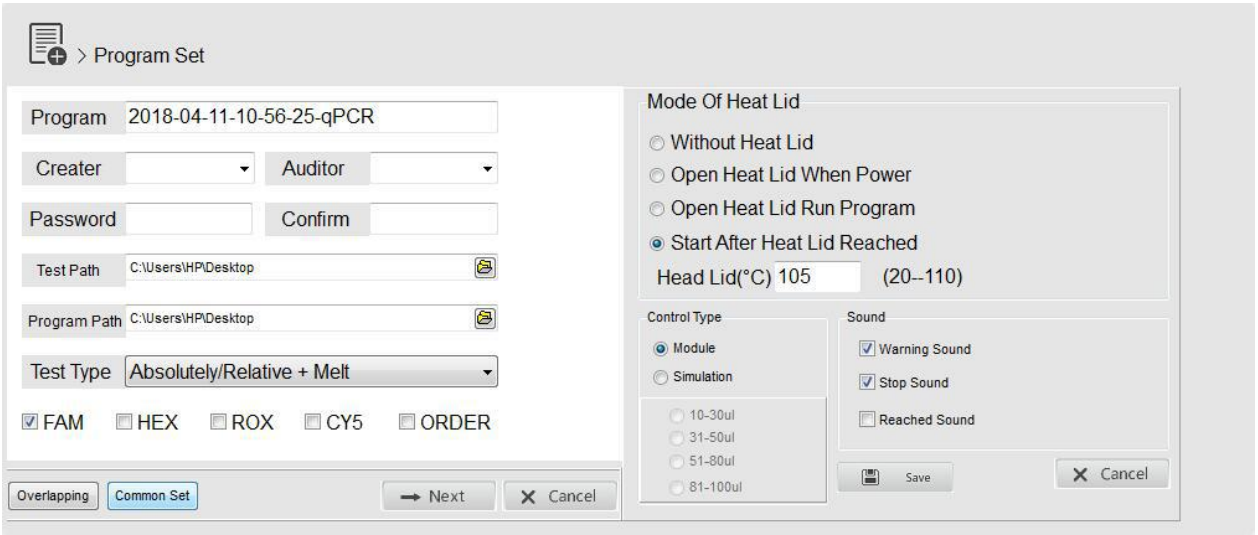
Test Type: Specify the type of current test.

Channel Selection: Select channels required for current test. At least one channel is required.

Cross-Interference: Call cross-interference setting page to compensate interference between multi-channel tests.



General Settings: Call general setting page to perform some basic settings.



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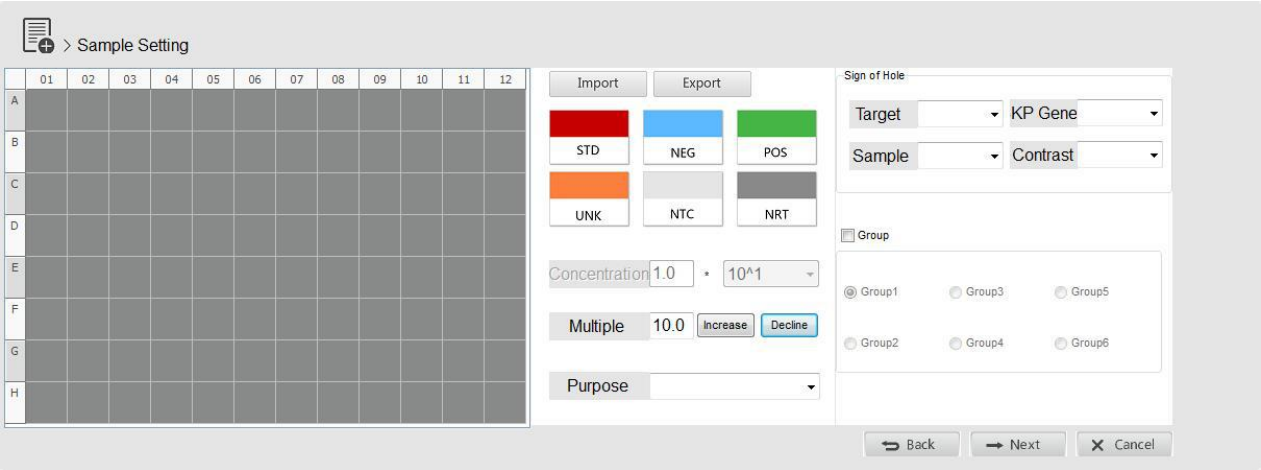
New Project - Sample Settings



Import: Import sample settings saved already.

Export: Export any sample information edited already for test next time.

Standard: Mark a certain hole position as Standard for a known concentration. This option should be used along with sample concentration.

Negative: Negative comparison type

Positive: Positive comparison type

Unknown: Sample type in unknown concentration

Gap: Gap comparison type, where water is generally used for comparison.

Unused: No sample is used for current hole position

Sample Concentration: Specify sample concentration for standard sample

Concentration Multiply/ Ascending/ Descending: Quickly mark hole positions arranging in rows or columns.

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Gene Name: Identify the project for the hole position

Sample Name: Identify the sample for the hole position

Housekeeping Gene: Identify housekeeping gene for current test

Standard Sample: Identify standard sample in contrast group for current test

Project Group: Divide projects into different groups

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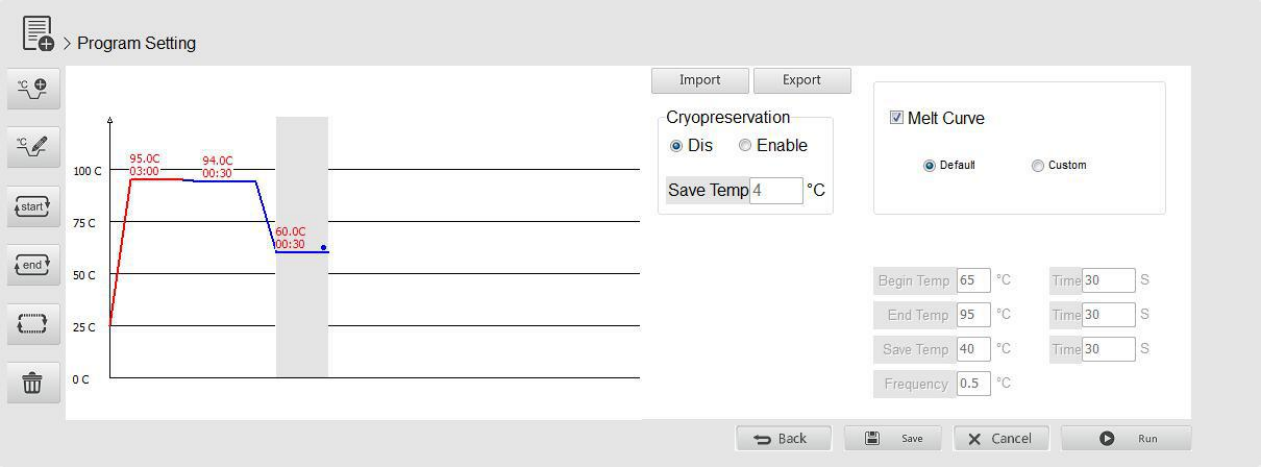
New Project - Sample Settings



Import: Import any program setting file saved already

Export: Export a program setting file edited currently

Low-Temperature Storage: For storage at low temperature upon completion of test

Solution Curve: Enable solution curve function

Save: Save current project file upon completion of test

Operate: Operate any project file saved when the instrument is connected and idle.

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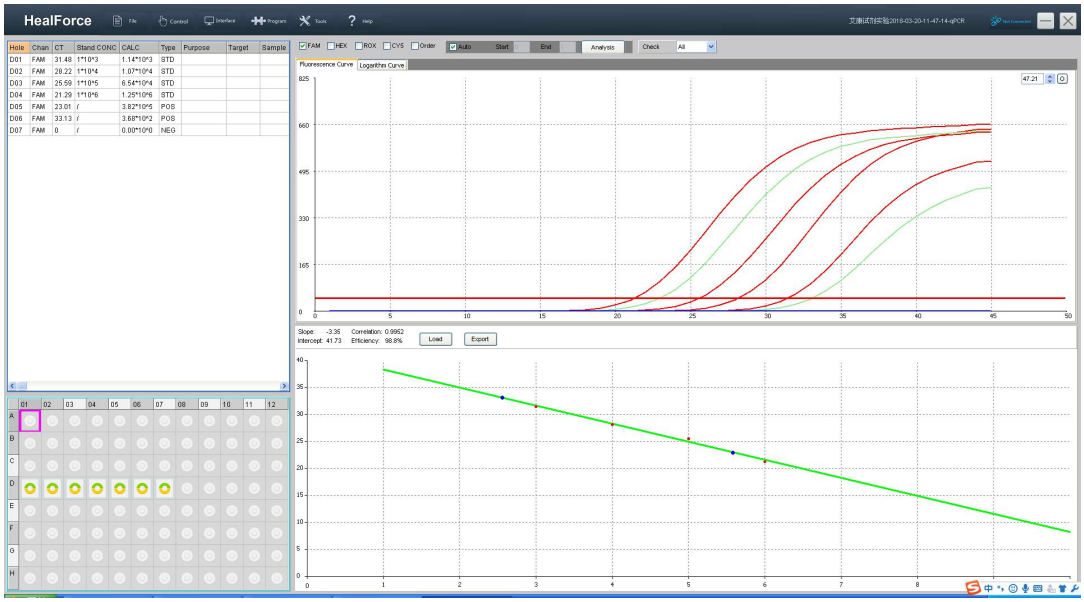
Analysis Interface



Introduction

It is used for analysis of test result after completion of test, including baseline setting, threshold analysis, outcome analysis, solubility curve, and quantitative analysis. .

Section V - Test Introduction and Description

Software Connection to Instrument

Step 1: Power the instrument on, and connect it to a PC by cabled or wireless means. Open the program when the instrument beeps, and the following information should be displayed. If not, refresh the instrument list by using right button.

Step 2: Click on Yes to connect it directly, or manually connect it in toolbar.

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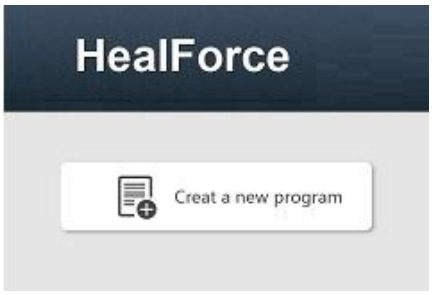
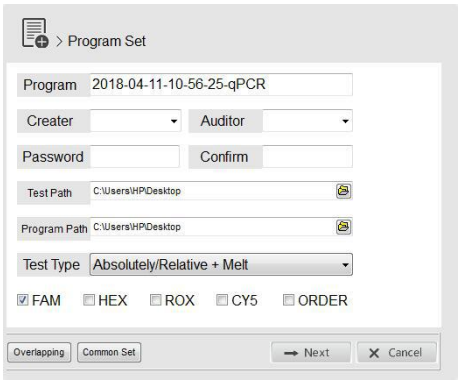
New Project File



Click on New Project in project wizard dialog box.



Enter basic setting page. Choose and enter proper settings and then click on Next.

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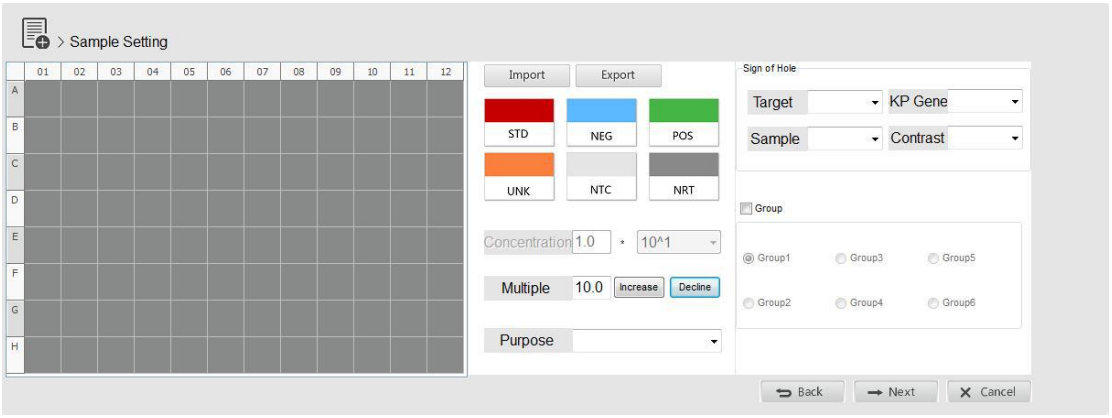
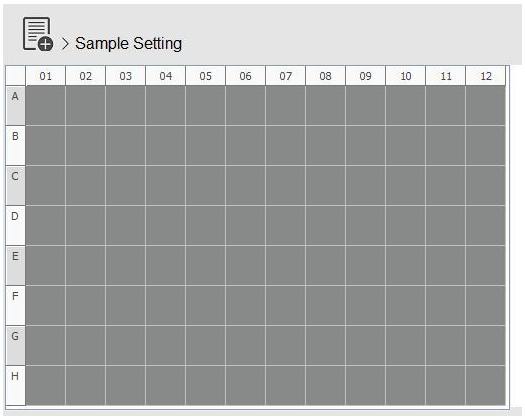
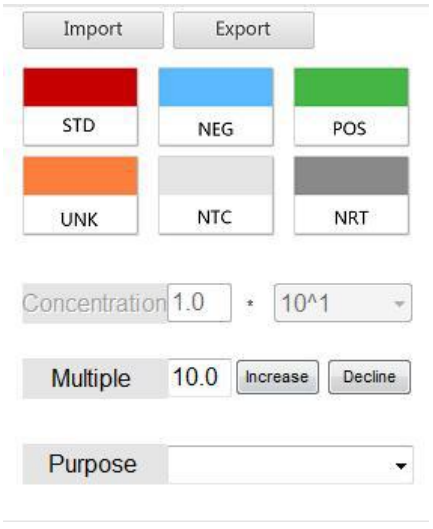

Sample Setting
01 02 03 04 05 06 07 08 09 10 11 12
A B C D E F G H
Import
Export
STD
NEG
POS
UNK
NTC
NRT
Concentration 1.0 * 10^1
Multiple 10.0 Increase Decline
Purpose
Sign of Hole
Target
KP Gene
Sample
Contrast
Group
Group1 Group3 Group5
Group2 Group4 Group6
Back
Next
Cancel



As required by the test, choose and mark desired hole positions for each test type.



In case of absolute quantitative test, hole position must be marked, or otherwise standard curve will be unavailable, and there are at least three standard samples in gradient. You may import any existing hole setting file. Quickly mark hole positions arranging in rows or columns with concentration multiply/ascending/descending.

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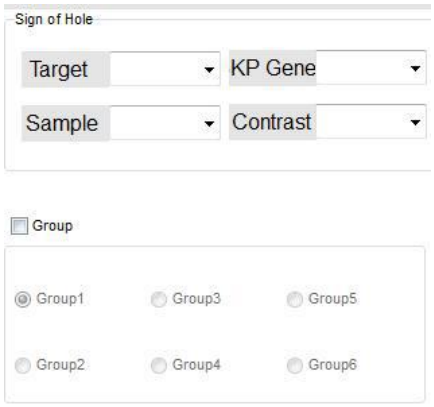
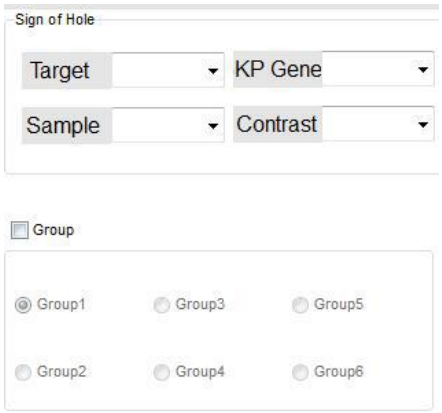
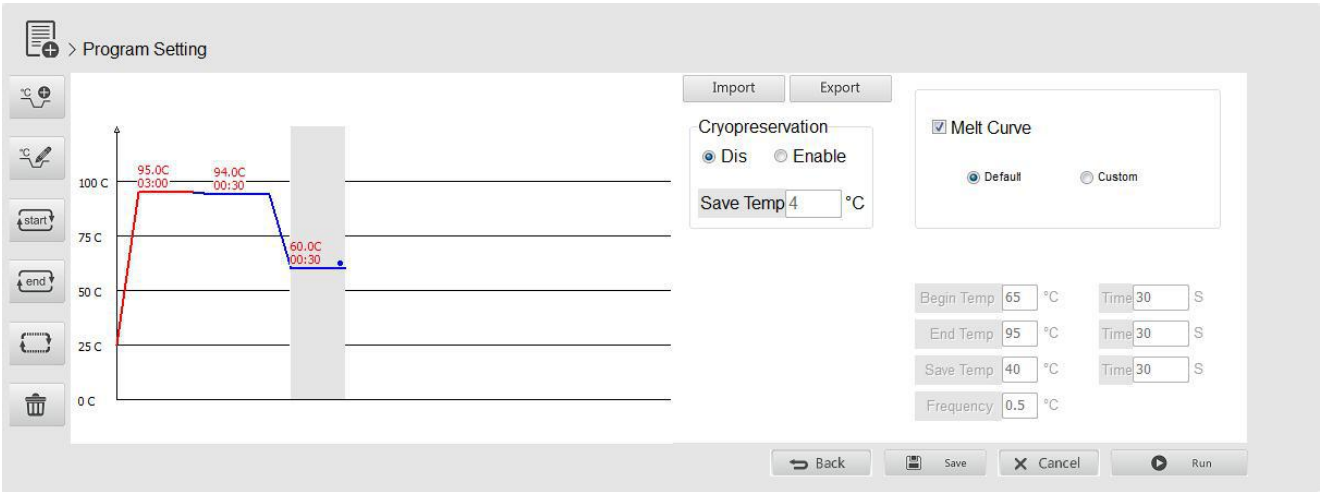


In case of relative quantitative test, gene and sample for each desired hole shall be identified, and housekeeping gene and reference sample shall be marked for the test.



When necessary, project groups can be identified for distinction.

Upon completion, click on Next



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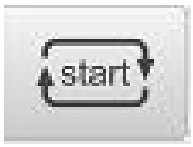
Button Function Description:

Add a temperature point

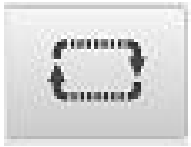
Change parameters for the temperature point chosen



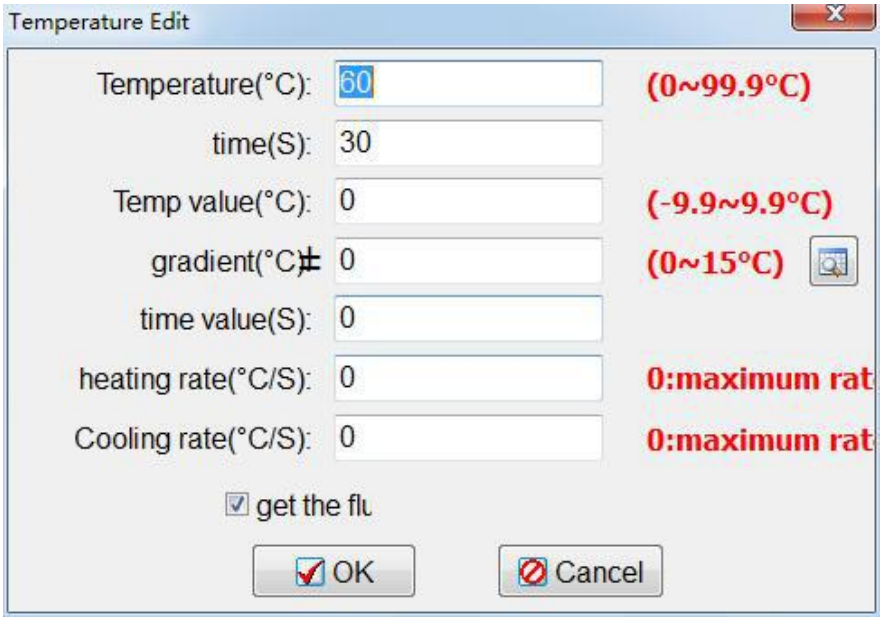
Set the selected temperature point as cycle start point

Set the selected temperature point as cycle end point

Delete a temperature point chosen



Change cycle number. Change or specify cycle number by dragging from a temperature point to an end point.



Temperature: Temperature set for a temperature point.

Time: Duration for a temperature point

Temperature variation: Increase temperature variation for each cycle, which will be reflected to the point in each cycle.

Gradient Width: Modular gradient can be used, and the whole module temperature will be distributed in gradient.

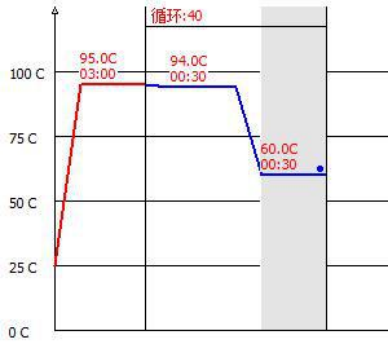
Time Variation: Increase time variation for each cycle, which will be reflected to the point in each cycle.

Heating Rate: Change heating rate, and the default is Fastest

Cooling Rate: Change cooling rate, and the default is Fastest

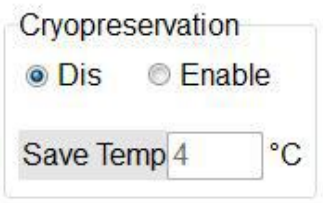
Collect Fluorescence: Mark it to collect fluorescence at current temperature point. Only one temperature point can be specified for each test. Set it by referring to related agent instructions.

Add at the end: Check it to add the temperature point to the end of the program.

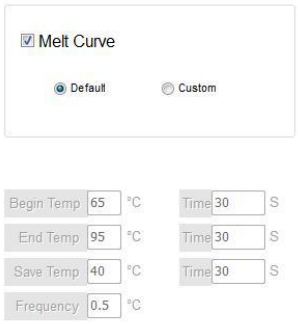


Created or changed temperature control program. The small blue point indicates the fluorescence strength in the agent finally collected at current temperature point.

Import existing temperature control program export a compiled temperature control program



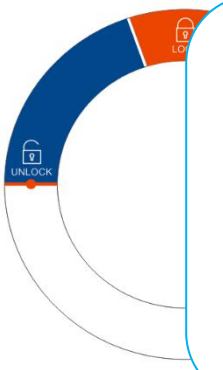
Set whether low-temperature storage is enabled and the storage temperature. This option is disabled by default.



Solubility Setting: It is recommended to use the default value. It is used to enable or disable solubility test, which is settable in customization. Save the project.

Specific Test Procedure

Locate reagent



First align the mark on the knob with point unlock, and then slide heated lid to put sample reagent into Hole 96 and then close the lid; rotate the knob clockwise to a to run the program for testing. It is recommended to align mark on the knob with the point LOCK.

Features of Real-Time Quantitative PCR

- Specificity: Quantitative PCR features both primer and probe, so is more special than conventional PCR.
- Sensitivity: Sensitivity of fluorescence PCR is up to 10^2 copies/ml, and the range of linearity in logarithmic phase analysis is 0~10^{11} copies/ml. Generally, the number of pathogens in a clinical specimen is 0~10^{10}/copy, within which quantitative PCR will dose accurately and the specimen need not be diluted.
- Repeatability: Quantitative PCR presents a stable result, since the threshold value is set in exponential amplification phase, during which, concentration of the reaction constituents is relative stable, free of side effect, and the logarithm of CT and fluorescence signal are in a linear relationship. Compared to Endpoint Method CT value is more stable and more accurately reflects copies of initial template.
- Safety: Without post-PCR operation steps, it reduces risk of pollution by the products.

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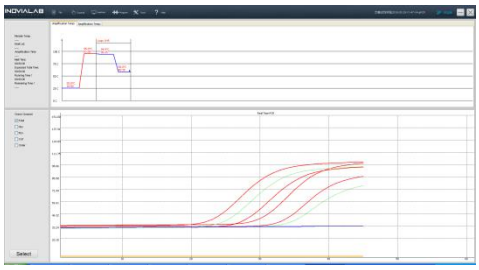
Running Program

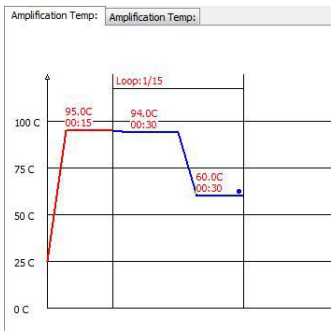


A project file can be run by either of two ways:

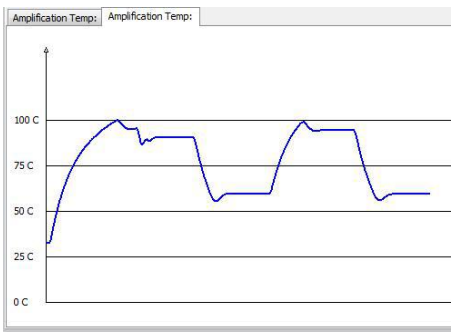
1.Click on Control > Run to run a project file saved in a folder.

2.Choose project management in project wizard and then choose the target program and right-click on Run.

After being started, the software module will display module information, lid information and estimated time.



After being started, the software module will display the increasing temperature for current project.



After being started, the software module will display the increasing temperature curve for current project.

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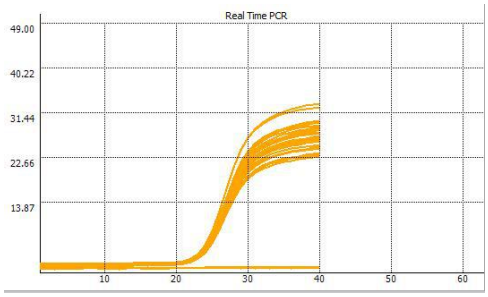
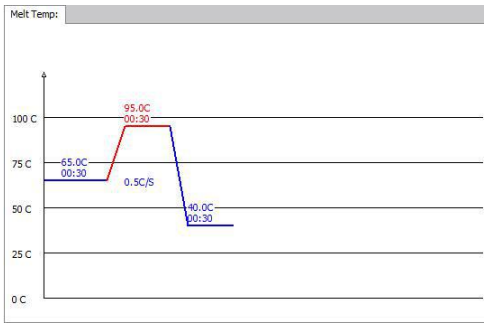
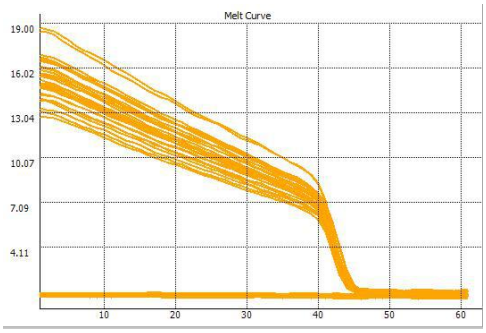


After being started, the software module will display the increasing fluorescence variation for current project.



When solubility curve is contained in the project, the software module will display current temperature variation.



When solubility curve is contained in the project, the software module will display current fluorescence variation.

As necessary, you can selectively review channels and holes

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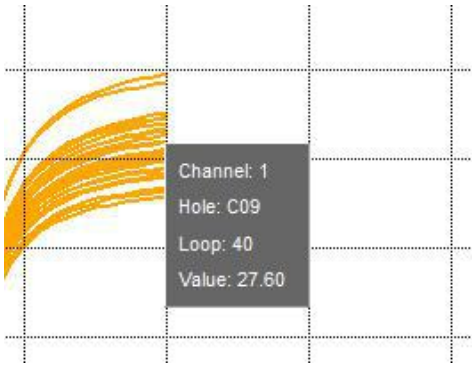
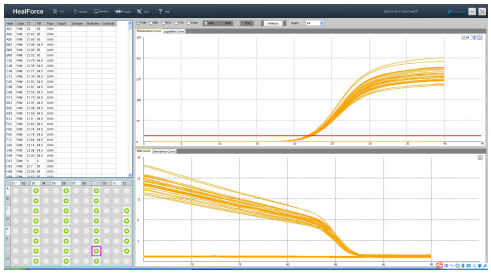
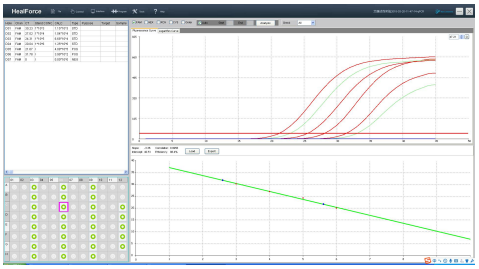


To view detailed output data for the fluorescence point, move the cursor to a point on the curve to pop up a box indicating detailed parameters.



Upon completion of test, the test file will be automatically saved to the directory as set for the project.



Test results are classified into result with CT value, absolute quantitative result with standard curve, relative quantitative result with histogram, SNP result with three pile diagram, and HRM result with differential diagram.

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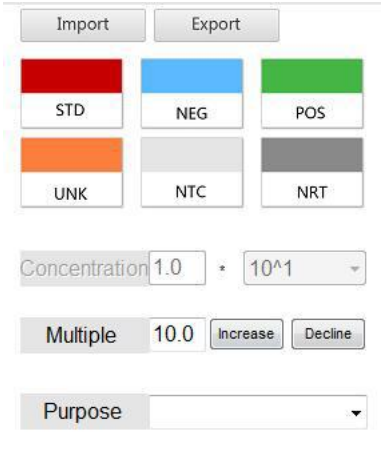
Test Analysis Operations

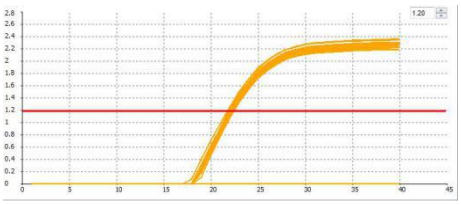
Principle and Application of Quantitative PCR

Real-Time Quantitative PCR is a method which adds fluorescence radical to PCR reaction system and perform real-time monitor of the whole PCR process by using fluorescence signals, and carry on quantitative analysis on the unknown template through s standard curve.

Click on Project > Change Parameters to choose a location where agent is located and mark it.



During operation, user can perform manual settings based reagent property in Reagent Parameters Setting at the right side, including: agent property, sample concentration, concentration multiple, channel, gene name marking, sample name marking, housekeeping gene settings, and standard sample settings.



In analysis interface logarithm curve, logarithm curve drawing is available and baseline can be re-positioned to achieve a more suitable CT value.

Baseline

Baseline is a straight line along which fluorescence signals vary slightly in the first several cycles of PCR amplification reactions.

In analysis interface, you can choose different channels, and make analysis by manually setting baseline or review curves of a certain type.

Hole	Chan	CT	TM	Target	Sample	Type	Group
A03	FAM	22.6	84.5	aaaa	1	UKN	0
A06	FAM	22.42	85	aaaa	4	UKN	0
A09	FAM	22.45	85	aaaa	5	UKN	0
B03	FAM	22.57	84.5	aaaa	1	UKN	0
B06	FAM	22.54	84.5	aaaa	4	UKN	0
B09	FAM	22.63	85	aaaa	5	UKN	0
C03	FAM	22.41	84.5	bbbb	1	UKN	0
C06	FAM	22.53	84.5	bbbb	4	UKN	0
C09	FAM	22.39	84.5	bbbb	5	UKN	0
C12	FAM	22.07	84.5	aaaa	6	UKN	0
D03	FAM	22.54	84.5	bbbb	1	UKN	0
D06	FAM	22.29	84.5	bbbb	4	UKN	0
D09	FAM	22.17	84.5	bbbb	5	UKN	0
D12	FAM	22.39	84.5	bbbb	6	UKN	0
E03	FAM	22.5	84.5	cccc	1	UKN	0
E06	FAM	22.53	84.5	cccc	4	UKN	0

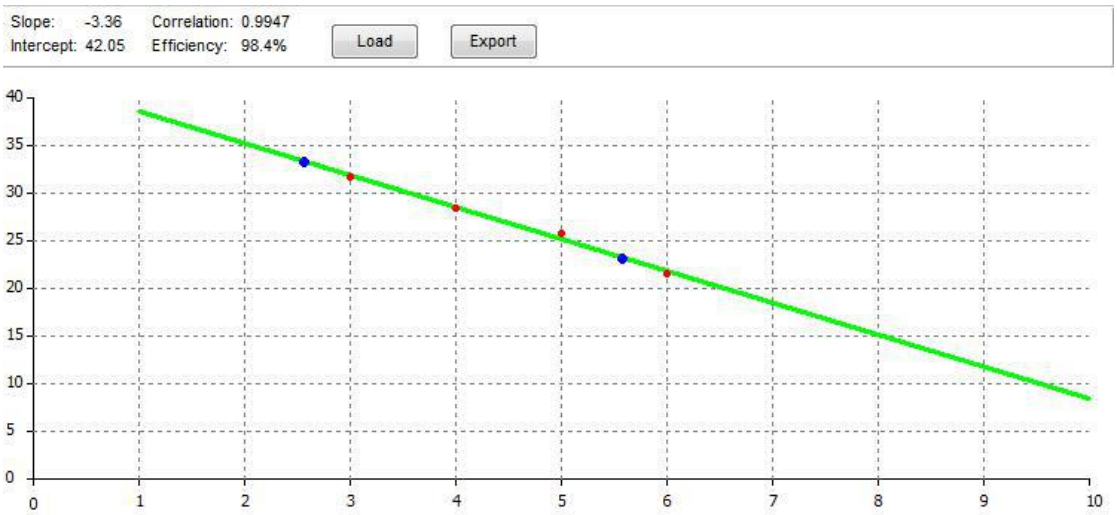
In this table you can view CT and TM values of a certain curve and other result information.

CT Value

Means the cycles which fluorescence signals in each PCR reaction tube undergo to reach the Threshold value as set. According to a research, CT value of each template is in a linear relation to the logarithm of initial copies of this template. The more initial copies, the fewer CT value, vice versa. A standard curve can be created by using a standard product in known initial copies, where the abscissa represents logarithm of initial copies, and the ordinate indicates CT value. Therefore, initial copies for an unknown sample can be calculated from the standard curve only by determining CT value of the sample.

Fluorescence Threshold Setting

Generally fluorescence signals in the first 15 cycles in PCR reaction are used as fluorescence background signals. Fluorescence Threshold value is 10 times of standard deviation of fluorescence signals in 3~15 cycles, and set in the exponential phase of PCR amplification.



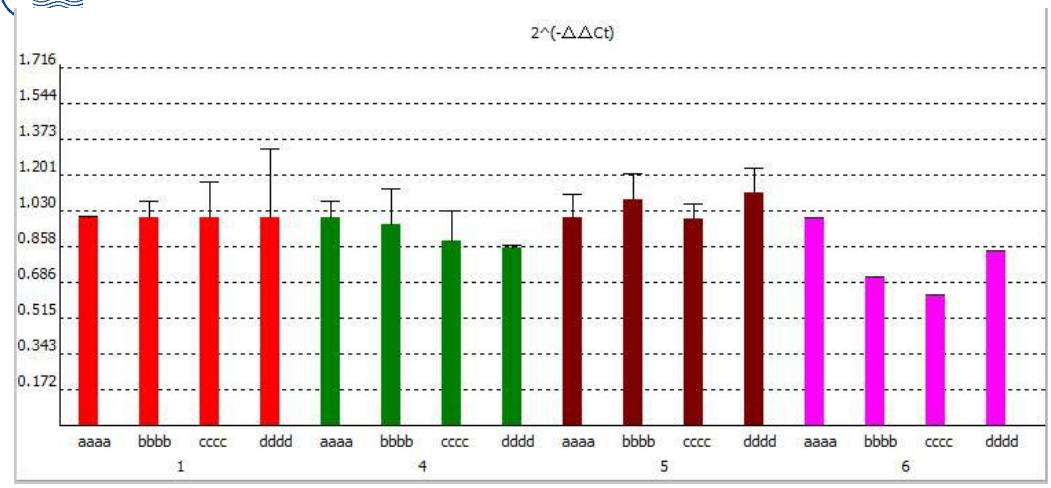
Absolute Quantitative Analysis - Standard Curve: Absolute Quantitative Analysis is available when standard samples are contained in test analysis and proper concentration in three or more gradients has been set for them.

The standard curve can be imported or exported in the interface.

Parameters Related to Standard Curve

- Standard samples are required in real-time absolute quantitative PCR test. The sample used to create standard curve can be in particle.
- Determination of molecular weight of standard sample： Concentration of DNA sample is 50 μg/ml when OD260 value is 1, based on the following formula:

  Concentration of sample particle (μg/μl) = OD260 value × nuclein dilution factor × 50/1000



Relative Quantitative Analysis - Analysis Method: $2^{-\Delta\Delta Ct}$ As the most classic relative quantitative analysis, it is mostly used in release of test report or result. Relative quantitative analysis is available when gene and sample names are specified in test analysis and housekeeping gene and standard samples have been set.

Description of relative quantitative test

- In relative quantitative test, gene and sample names need to be specified, and housekeeping gene and standard samples need to be marked. In lab test, this method is mostly used in final test analysis.
- Where error analysis is required, each gene and sample need to be re-tested based on the condition above.

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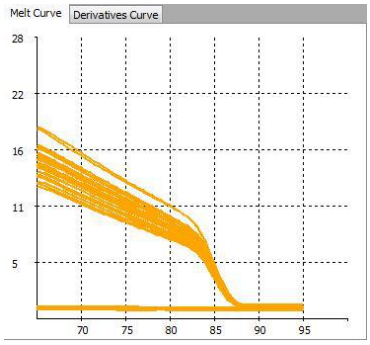
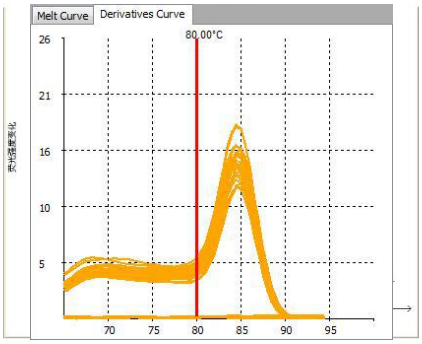


Absolute light intensity in solubility analysis: Where solubility test is contained in initial test, the software will draw absolute light intensity for current solubility test, and the testing personnel will visually observe variation of fluorescence intensity during test.



Relative light intensity in solubility analysis: As determined from the absolute light intensity above, fluorescence value variation trend is clearly observed during solubility test. The red line in the diagram will help testing personnel in positioning the temperature point with the most intensive variation of fluorescence strength.

Hole	Chan	CT	TM	Target	Sample	Type	Group
A03	FAM	22.6	84.5	aaaa	1	UKN	0
A06	FAM	22.42	85	aaaa	4	UKN	0
A09	FAM	22.45	85	aaaa	5	UKN	0
B03	FAM	22.57	84.5	aaaa	1	UKN	0
B06	FAM	22.54	84.5	aaaa	4	UKN	0
B09	FAM	22.63	85	aaaa	5	UKN	0
C03	FAM	22.41	84.5	bbbb	1	UKN	0
C06	FAM	22.53	84.5	bbbb	4	UKN	0
C09	FAM	22.39	84.5	bbbb	5	UKN	0
C12	FAM	22.07	84.5	aaaa	6	UKN	0
D03	FAM	22.54	84.5	bbbb	1	UKN	0
D06	FAM	22.29	84.5	bbbb	4	UKN	0
D09	FAM	22.17	84.5	bbbb	5	UKN	0
D12	FAM	22.39	84.5	bbbb	6	UKN	0
E03	FAM	22.5	84.5	cccc	1	UKN	0
E06	FAM	22.53	84.5	cccc	4	UKN	0

As shown in information on solubility test, testing personnel can quickly search for specific TM value for each hole in the table.

Description of solubility test

- In QPCR test by dye method, solubility test is generally required. Test by dye method has poor specificity, so solubility test will be performed to validate whether primer dimer which is not concerns of the test is produced in test.

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Instructions on Amplification Curve

Determine whether an amplification curve is acceptable based on the following:

- Clear knee points, particularly distinctive low-concentration sample exponential phase. Parallel amplification curve as a whole, flat baseline without rise, and distinctive low-concentration sample exponential phase.
- Slope of exponential phase in direct proportion to amplification efficiency - the more slope, the higher amplification efficiency.
- Standard flat and straight baseline or with slight descent, but without obvious

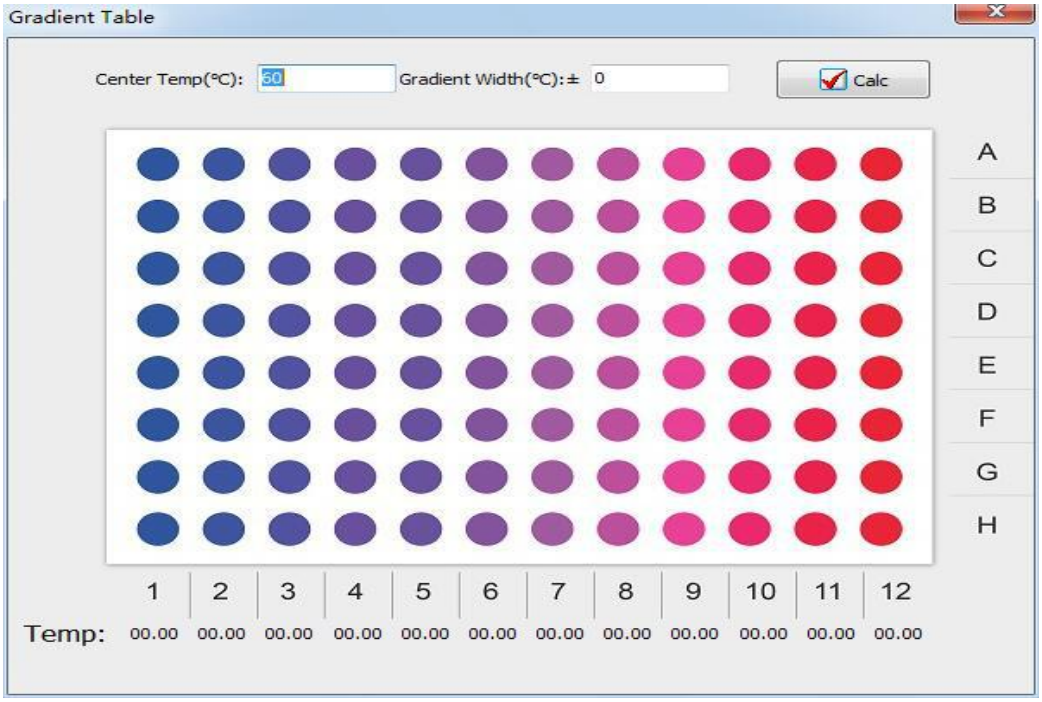
Test Considerations

- Rules and regulations issued by the authorities shall be complied with by the laboratory.
- Fluorescent material-free disposable gloves, disposable dedicated centrifugal tube, self-removing pipette and suction head with filter element shall be used.
- Prepare and handle reaction fluid in such way to avoid generation of air bubble; check whether reaction tube is sealed tightly prior to startup to prevent leakage of fluorescent material and thereby pollution to the instrument.
- During loading sample soak the sample entirely into the reaction fluid and prevent attachment of any sample onto the tube wall; seal the tube after loading.
- Take the reaction tube out immediately after completion of amplification, and seal it in dedicated plastic bag and keep the bag at specified location for approval and treatment.

- Suction head used in test shall be put in waste jug containing sodium hypochlorite of 1%, and reject it after sterilization with other waste.
- The test platform and all test appliances shall be disinfected with sodium hypochlorite of 1%, alcohol of 75% or UV lamp.
- PCR reaction mixed liquor shall be stored at low temperature and protected from sunlight.
- The test platform shall be cleaned after completion of test to avoid contamination.

Section VI - Other Function Introduction

Grade Calculation and Retrieval



About Grade Functions

Click on "Grade" from main menu to access View Grade page. For convenience of user, the software provides a grade table for user's retrieval. Upon entry of central temperature and gradient width, the software will automatically calculate specific temperature value for each column and helps to ensure accurate test result.

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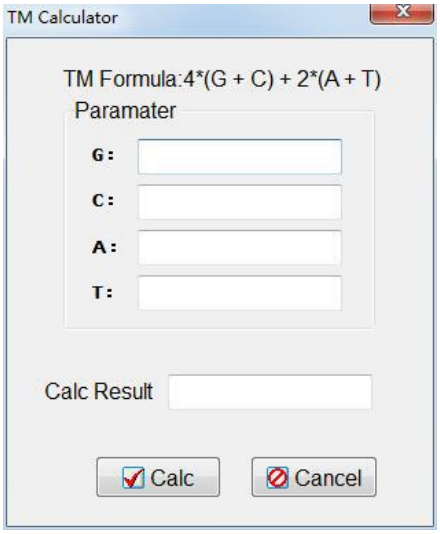
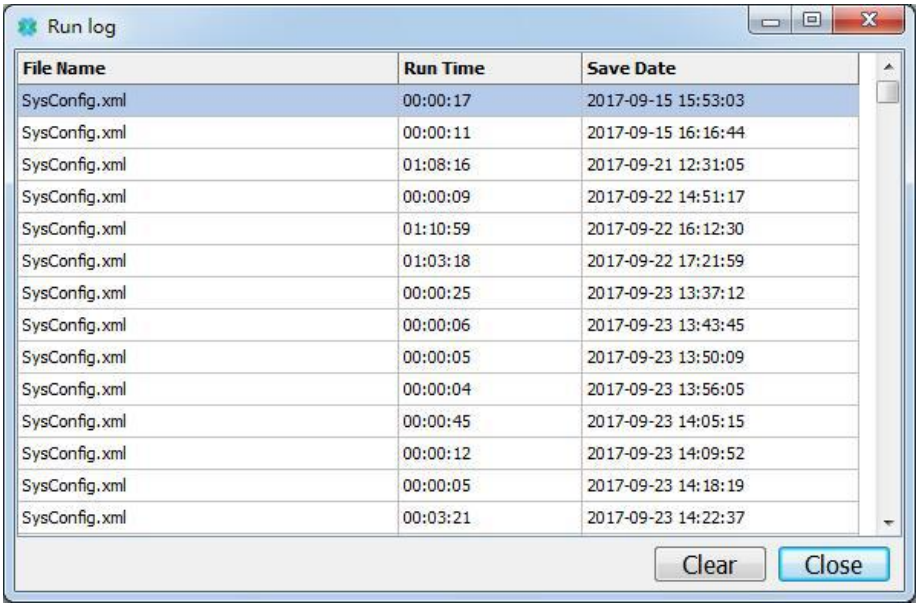
Auxiliary TM Value Calculator



About TM Value Calculation

- Click on "TM" from main menu to access TM Calculation page. Enter a value and then click on "Calculate" to output the result.
- The program is furnished with an embedded TM value calculator for user's access when necessary.

Logs Recording and Retrieval



About Logs

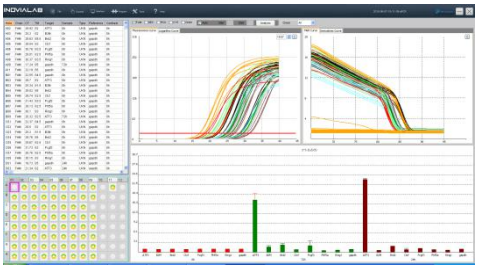
- Click on "Logs" from the main menu to access Logs page and view test operation logs.
- Facilitate management of test history records by user and retrieval of related test history records.

Other Internal Operational Functions

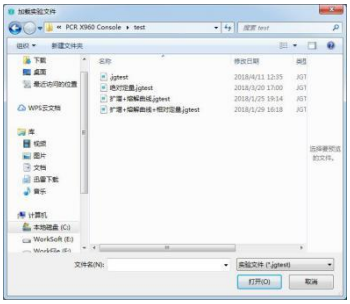
Chinese-English Toggle Button

Click on Tool > Chinese or English to change the language.

Load test data.



Click on “File > Load Test” in main menu.



In the popup dialog box, select the target data (in format of .jgtest) stored previously to load.

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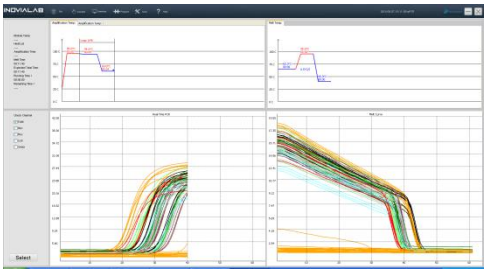
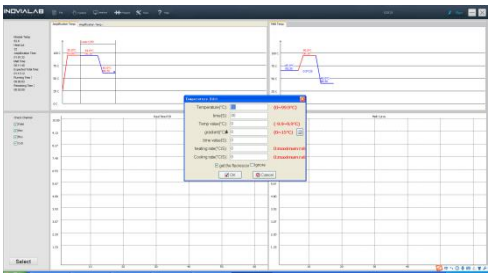
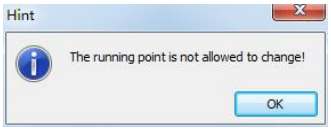


After loading the data, you can perform relate test analysis on the loaded data.

Real-Time Modification to Operation Parameters



During operation of the program, double click on a temperature segment to modify a parameter in the popup dialog box.



Modification can be made only by double clicking on a temperature segment currently not in operation, or otherwise a message box will occurs, indicating "No modification can be made to any node currently in operation".

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