

Operation Manual
DTprime
Real-Time PCR instrument
PART I
Operating Procedure

TS 9443-004-96301278-2010



DNA-Technology, Research & Production LLC

Protvino



Safety Information

IMPORTANT: READ THIS MANUAL CAREFULLY BEFORE USING THE INSTRUMENT

General Safety

Whenever see the sign  **Caution! Danger! refer to the manual.**

Before using the “DTprime” Real-Time PCR instrument (hereafter – the “instrument” or “device”), please read this section carefully and pay particular attention to the safety information. To avoid accidents, as well as breakdown of the instrument and equipment used therewith, observe the safety rules given below.

If safety rules are violated during exploitation or in case of unintended use of the instrument, the protection provided by the instrument may worsen and a health hazard may arise.

Do not operate the instrument if humidity exceeds 80% in the room. Condensate formation may result of a short circuit in the instrument electronics.

The instrument should be saved from shocks and falling.

The instrument must be stored and transported only in a vertical position.

After transport or storage in humid and /or cold conditions, dry out the instrument not less than 3 hours before connecting it to the supply voltage.

Avoid any liquids or objects getting inside the instrument's cabinet. This may result in breakdown of the instrument.

“DNA-Technology, Research & Production”, LLC bears no responsibility for any injuries or damage to health caused by unintended use or unauthorized modifications of the instrument.

Electrical Safety

Prior to connecting the instrument to an electric grid, check the presence of protective grounding in the socket to which the instrument will be connected as well as integrity of the power cable. **Connection to the socket without grounding is prohibited.** Please use the power cable provided in the set of delivery. If liquid is spilt inside the instrument, disconnect it from the power supply immediately and contact local service center or distributor.

Please connect the instrument to an electric grid with the corresponding voltage, which is mentioned on the identification plate of it.

 **Caution! Danger of electrical shock! Replacement of fuses of the instrument has to be carried out in compliance with the rules and guidelines of electrical safety regulations.** Fuses have to be replaced only when the instrument is switched off. The instrument is considered to be unplugged if it is unplugged of the electricity socket and the USB cable is disconnected from the USB 2.0 port of a PC.

During Operation

Do not expose the instrument to heat and sunlight or bright light from other intensive light sources.

Maintenance

Do not open the cabinet of the instrument! It does not contain user serviceable components.

Note! Settings of motor controllers, calibration parameters of optical and temperature modules of the instrument cannot be changed by the end user. Calibration of the instrument has to be performed by the manufacturer in accordance with the internal regulations of quality control. Calibration data, if necessary, can be provided by the service department of DNA-Technology Research& production

Operational Safety

The instrument is designed to be used under the following environmental conditions:

- indoors;
- altitude up to 2 000 m;
- temperature from 5 °C up to 35 °C;
- maximum relative humidity of 80% for the temperature up to 25 °C;
- main voltage from 100 up to 220 V, frequency 50/60 Hz.

The instrument is IVD medical equipment and it is intended for studying of DNA samples using the polymerase chain reaction (PCR) method and the detection of the accumulated product in real time (real-time PCR). The instrument does not carry a direct biological hazard. Servicing and maintenance of the instrument should be performed by specially trained, qualified staff.

While operation with the instrument:

- wear suitable protective clothing, gloves and secure eyes and face;
- do not pipette solutions by mouth;
- do not eat, drink, smoke or use cosmetic products in the operational area;
- after handling samples and chemical reagents wash your hands carefully;
- dispose of leftover / used reagents and waste in compliance with the regulations in force;
- read the instruction for use provided with the PCR kit before running the assay;
- while running the assay, follow the instructions for use;
- do not use a PCR kit after its expiration date;
- use only the chemical reagents provided along with the kit and those, which are recommended by the manufacturer;
- do not mix chemical reagents from different PCR kits or lots.

Environmental Effect

Deinstallation of the instrument for maintenance or recycling purposes: if properly operated, the device doesn't bear biologically hazardous.

Recycling of the instrument should be carried out in accordance with the regulations in force at the area. The device does not contain any substances, which may represent a direct threat to the environment.

Notice

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Table of Contents

1. General Information	8
1.1. Intended Use and modifications of the instrument	8
1.2. Delivery Set	9
1.3. Technical specification	9
1.4. Operating Conditions.....	11
1.5. PC Requirements	11
2. Instrument description and principle of operation.....	12
2.1. Fast Thermal Control System	14
2.2. Optical system	14
2.3. Control and Display System	15
2.4. Marking. Symbols used for marking.	16
3. Setting up the instrument.....	18
3.1. Instrument Unpack	18
3.1.1. Instrument Carrying.....	18
3.2. Instrument installation	18
3.3. Software Installation.....	19
3.3.1. Instrument Driver Installation.....	19
3.3.2. Installation of RealTime_PCR software.....	25
4. Operating Procedure.....	29
4.1. Instrument Switching-on and RealTime_PCR Launch	29
4.2. Preparing the Instrument for PCR	30
4.2.1. Checkup of Geometrical Settings	30
4.2.2. Optical Measurement Exposure Setting	31
4.2.3. Checking the Cleanness of Thermal Plate Wells.....	32
4.2.4. Tubes Height Measurement.....	32
4.3. Creation of Plate protocol and Amplification Program	33
4.3.1. Filling in of Plate protocol.....	33
4.3.2. Creation/editing of Amplification Program.....	34
4.4. Placement of Tubes with Samples.....	35
4.5. Startup of the amplification program.....	37
4.6. Amplification program running.....	38
5. Maintenance	40
5.1. General Guidelines	40
5.2. Maintenance Procedure	41
5.3. Disinfection requirements of the instrument prior to maintenance and repair procedures.....	41
5.4. Troubleshooting.....	42
6. Storage and shipment	42
7. Recycling.....	43
8. EMS declaration	43
9. Warranties.....	44
10. Packing Certificate	45
11. Acceptance certificate.....	45
12. Cards of warranty repair	47
13. List of claims and instrument disinfection procedure.....	49

Introduction

The “DTprime” Detecting Thermocycler (hereafter – the “instrument” or “device”) is intended to conduct qualitative and quantitative PCR analysis dropping out electrophoresis stage of PCR products in agarose gel when using PCR kits based on the fluorescent detection principle. Measurement of the quantity of accumulated product of PCR amplification takes place right in the course of reaction temperature cycles (real-time PCR). The qualitative analysis is based on evaluation of kinetics of PCR visible part, carried out using relevant mathematical tool.

The use of fluorescent detection of PCR products has a list of substantial advantages:

- high detection specificity (if oligonucleotide probes are applied, which allow detecting only of certain amplicons);
- high efficiency;
- reduced assay time;
- carrying out detection in a closed tube, which practically eliminates contamination of further experiments;
- availability of quantitative assessment of initial DNA-matrix;
- registering and accounting of the data in electronic format.



Fig.1. Instrument General View

1. General Information

1.1. Intended Use and modifications of the instrument

The “DTprime” real time PCR instrument is intended for carrying out an assay using polymerase chain reaction (PCR) method and accumulated product detection in a real time (real-time PCR). Detection of the accumulated PCR amplification product occurs directly during the execution of the amplification program, which increases the informative value of the study.

The field of application - clinical laboratory diagnostics in vitro;

Purpose of use – diagnostics;

Sample type - samples of human DNA and microorganisms extracted from samples of biomaterial;

The instrument is manufacturing in the following modifications: 2M1, 2M3, 2M6, 4M1, 4M6, 5M1, 5M3, 5M6, 6M6, 2X1 4X1, 5X1, 6X1.

Modification of the instrument is defined by the following structure – XXX, where:

- 1) 1st X is figure from 4 to 5 and it indicates the number of optical detection channels;
- 2) 2nd X is the symbol M or X and it determines the thermal block format: M - 96 wells of 200 µl, X - 384 wells of 45 µl.
- 3) 3rd X is figure 1, 3 or 6 and it means a thermal block version such as 1 – monoblock; 3 or 6 - the number of thermal block sections.

The instrument is designed for simultaneous analysis of up to 96 or up to 384 samples depending on the modification (M or X).

Preparation and initialization of experiments on the instrument is realized with an IBM-compatible personal computer, and further implementation of a preset program may be executed autonomously. On completing the amplification program, the instrument goes to the storage mode, and if the option “**Turn off device after the run**” is activated on the window “**Running**”, the instrument goes to the power standby mode.

The optical measurements results may preliminarily be evaluated during the execution of the amplification program, whereas the final evaluation – at any time after completing the program. All data of the last amplification are saved in the internal memory of the instrument and may be read out even in the case of power failure during amplification. As soon as the mains supply is restored, the instrument continues to function with full recovery of the amplification program status. Neither failure of the operating system, nor disconnection of the external computer will halt the experiment. Thus, the instrument is highly protected against major factors effect, which may result in a failure during PCR implementation.

One computer may control operation of several instruments, including ones of different types (DTlite, DTprime) connected to it via USB ports.

To operate the instrument, the RealTime_PCR software is used, which allows performing the following operations:

- Test creation and editing;
- Plate protocol creation;
- Checking geometrical and optical settings of the device;

- Creation, launching, and monitoring of the amplification program;
- Monitoring the execution of the amplification program
- Analysis of optical measurements data.
- Data analysis report.
- Settings and diagnostics of the instrument.

Loading and unloading of analyzed samples may be automatic in case if the instrument is built in a robotic system.

Instrument is open system and it is not depended of concrete PCR kits.

1.2. Delivery Set

No.	Description	Reference	Producer	Quantity pcs.
1	DTPPrime real time PCR instrument	RT96-01IN	DNA-Technology R&P	1
2	Instruction for use (Part I and Part II)	RT96-04IFU	DNA-Technology R&P	1
3	Computer communication cable USB 2.0 High Speed A-B	—	China	1
4	Power cable (10A, 250V, 3x0,75mm ²)	—	Taiwan	1
5	Fuses. (10A, 5x20, 250 V)	179200.10	Germany	1
6	Distribution package of software	RealTime_PCR version 1.0.0.198 and higher	DNA-Technology R&P	2
7	Packing unit	RT96-03PG	DNA-Technology R&P	1

The instrument may be delivered along with portable PC—with the pre installed software in accordance with customer's request. Note: PC should be purchased along with the order of the instrument.

1.3 Technical specification

Specification	Value
Thermal block format (modification M)	96 test tubes of 0.2 ml (12 x 8)

Thermal block format (modification X)	384-well microplate
Tube type (modification M)	0.2-ml test tubes for PCR (individual, in strips, 8 pieces each, or in plates 12 x 8)
Tube type (modification X)	384-well microplate, 24x16 wells of 45 µl volume
Reaction volume (modification M)	10-100 µl (10-50 µl recommended)
Reaction volume (modification X)	5-30 µl
Temperature range	0 °C-100°C
Temperature step	0.1°C
Temperature accuracy	±0.2°C
Temperature uniformity	±0.15°C
Average heating ramp rate within temperature range of 0...100°C (modification M) Maximum heating ramp rate within temperature range of 0...100°C (modification M)	3.3°C/s 3.5°C/s
Average cooling rate within temperature range of 100...55°C (modification M) Maximum cooling rate within temperature range of 100...55°C (modification M)	2.1°C/s 2.5°C/s
Average heating ramp rate within temperature range of 0...100°C (modification X) Maximum heating ramp rate within temperature range of 0...100°C (modification X)	2.1°C/s 2.5°C/s
Average cooling rate within temperature range of 100...55°C (modification X) Maximum cooling rate within temperature range of 100...55°C (modification X)	1.0 °C /s 1.5 °C /s
“Hot lid” temperature	105°C ±1°C
Heating and cooling method	Peltier elements
Excitation source	LED
Detector	CCD camera

Number of optical channels	4 (versions 2M1, 2M3,2M6,2X1) 4 (versions 4M1, 4M3,4M6) 5 (versions 5M1, 5M3,5M6, 4X1,5X1) 4 (versions 6M1, 6M3,6M6,6X1) *
Excitation/Detection wavelengths for each channel, nm	470/515, 530/560, 580/620, 630/660,687/731(*)
Threshold sensitivity of each channel for standard fluorophors solutions	0,05xE ⁻¹² gram-mol
Data connection	USB 2.0 High-speed
Power consumption	550 W (max.)
Mains voltage	220 V
Line frequency	50/60 Hz
Dimensions, WxDxH	210x540x540 mm
Starting operation after switching on	no more than 5 minutes
Weight	27 kg

* spectral characteristics of optical channels can be changed within the specified limits 450 ÷ 750 nm in accordance to customer request.

1.4. Operating Conditions

The instrument is intended for the use indoors under the following climatic conditions:

- indoor temperature should be from 5°C to 35°C;
- relative humidity for temperatures up to 25 °C should not exceed 80%;
- at an altitude of not more than 2000 meters.

1.5. PC Requirements

The instrument operates under the control of IBM-compatible personal computer with the following minimum requirements:

- 1 GHz Pentium IV;
- 512 Mb RAM;
- Free hard drive space 100 Mb;
- Screen resolution 1024x768 pixels;
- USB 2.0 High-Speed port.

To control the instrument the following parts of software pack must be installed:

- the instrument driver;
- Realtime PCR software to control the instrument and perform data analysis.

The software may be downloaded for free from the company's official website at <http://www.dna-technology.ru/eng/support/>

The software is compatible with the following operating systems: Windows 98, Windows ME, Windows 2k/XP, Windows Vista, Windows 7, Windows 8, Windows 10.

2. Instrument description and principle of operation

DTprime is a specialized instrument combining a precise programmable thermal cycler and an optical system to collect fluorescent data at each stage of polymerase chain reaction.

Design of the instrument:

The design of the instrument is a supporting frame which bears the following installed modules:

- thermo block including positioning module of actuated parts of the instrument designed for automatic moving of it;
- the optical system module which consists of sources of excitation based on LEDs and detection based on CCD camera;
- optical path module for transporting light beams;
- slide for the thermal block placement;
- hot lid module, which presses the tubes from the top and heat them for preventing condensation and also eliminates self-opening of the tubes in order to avoid a possible contamination of the instrument by amplicones;
- horizontal drive, which provides movement of the thermal block in horizontal plane for loading and unloading tubes into it;
- vertical drive, which provides movement of the thermal block in vertical plane to ensure that the tubes are pressured to the thermal lid;
- electronics modules including power supplies;
- cabinet of the instrument, which has several ventilation holes.

The instrument face panel accommodates the following (fig 2):

- LCD panel;
- push-button control panel;
- “hot lid” front panel;
- thermal block front panel.

Side walls of the instrument have ventilation slots.

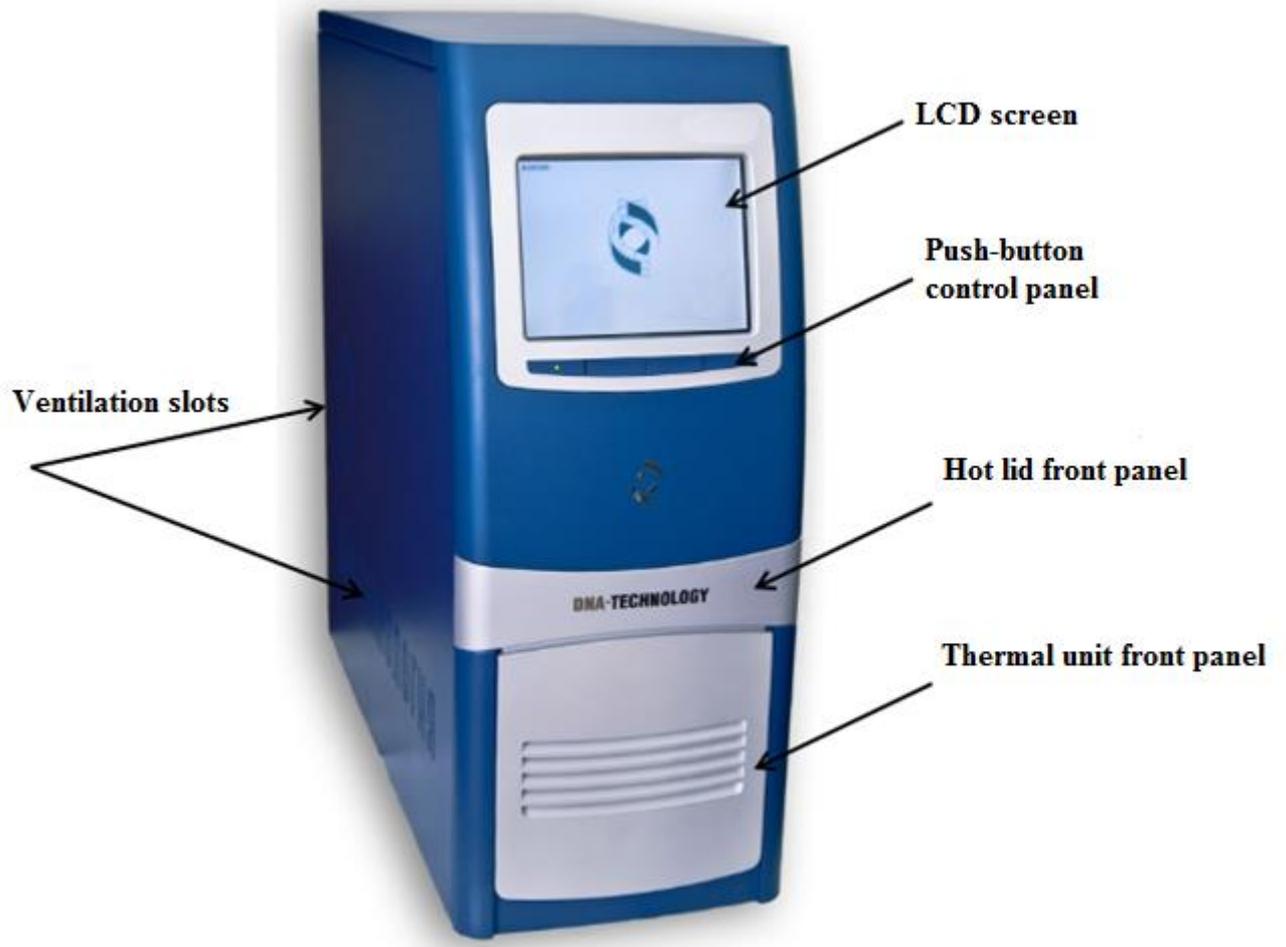


Fig.2. Front view of DTprime instrument

The instrument rear panel accommodates the following (fig 3):

- power switch;
- two fuses;
- power input;
- USB connection (type B).

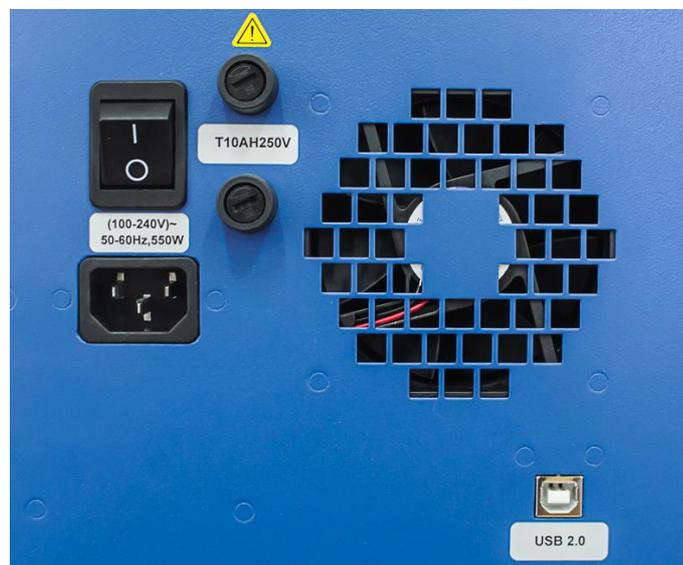


Fig.3. Rear view of DTprime instrument

Functionally there are three main systems of the instrument:

- ❖ high-rate temperature control system;
- ❖ optical system;
- ❖ control and display system.

2.1. Fast Thermal Control System

The system of high-rate temperature control includes a thermal unit, its movement and positioning module, and the hot lid module.

The thermal block ensures thermal cycling of the tubes according to the amplification program. Six Peltier elements are used for thermal plate cooling and heating providing high accuracy of temperature regulation, noiselessness, good characteristics of mass-and-dimension ratio and high reliability. Each of these Peltier elements has its own temperature sensor and regulator that allows the preset of necessary temperature gradients across the plate.

The instrument thermal plate is made of aluminum alloy, which possesses an optimal ratio between good thermal conductivity, weight, and processing characteristics. The plate design is a cellular structure with reinforcing ribs and corresponding number of wells:

- in M modification – 96 wells for standard 200 mcl PCR tubes with distance between the rows as in standard 96-well plates. The well shape provides optimal heat transfer to the samples with volume up to 50 mcl.
- in X modification – 384 wells for standard 384-well PCR microplates.

Note! For stable results, tubes with the angle of tapered part of $17^{\circ}20'$ must be used.

Movement and positioning module is intended for precise positioning of the thermal unit within 180 mm and 50 mm in horizontal and vertical planes correspondently and provides easy access to the tubes.

The hot lid module provides a reliable heat contact between tubes and the thermal plate and allows maintaining temperature of the tube caps at the level of $105 \pm 1^{\circ}\text{C}$ in order to prevent condensate formation on the tube caps, since it may cause substantial distortions in the luminous flux measurements. The hot lid design provides an adjustable force of tube-to-plate hold-down that depends on the number of the tubes installed. During running PCR the thermal block moves vertically from bottom to top. The moment of contact of the tubes with the hot lid is monitored by a special sensor, which passes information to electronic module of the instrument to calculate the necessary pressing force. The positioning module moves the thermal block vertically by an amount that provides the necessary pressure to hold the tubes against the surface of the hot lid.

2.2. Optical system

The optical system consists of an optical module and a light beam transmission module.

The optical module is a system of lenses, mirrors and light filters that provides folding of the luminous flux from several light sources on one optical axis and its separation from the luminous flux entering the CCD camera. The light sources are powerful LEDs. The light source design provides efficient heat removing from emitting crystals of the light-emitting diodes for stable luminous flux.

Optical path (light beams transmission module) ensures the transmission of the luminous flux from light sources of the optical module to the tubes, and back transmission of the fluorescent luminous flux from the tubes to CCD camera.

The module for light beams transmission is a rectangular duct. Rectangular mirrors located in the duct corners provide the optical pathway in a required direction.

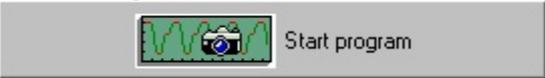
The system of light locks provides an absolute isolation of the optical channel from external light.

2.3. Control and Display System

The control and display system comprises a push-button panel for manual control of the thermal block movement and the LCD screen indicating the process of amplification program running (Fig. 4).

Push-button of on-screen menu displays functions of other panel push-buttons within the given operation mode. **The push-button of thermal block control** (the second one from the left) is intended for launching the following operations:

- opening and closing of the thermal block for placing the tubes into the thermal plate before PCR running;
- precise positioning of the thermal block under optical elements in their operating position.

When the instrument operates under computer control, one can open or close the thermal block using  and  buttons located under the button  in the **Running** window of RealTime_PCR software.

LCD screen displays information about an amplification program and the running process.

It shows the following information:

- operator's name;
- date of the amplification program start;
- Plate protocol number;
- name of the amplification program;
- instrument serial number;
- quantity of conducted measurements;
- amplification program timer;
- instrument status on completion of an amplification program;
- current temperature of the reaction mix;
- description of the amplification program;
- fluorescence measurement channels enabled;
- optical measurement indicator;

- indicator of the amplification program run status.

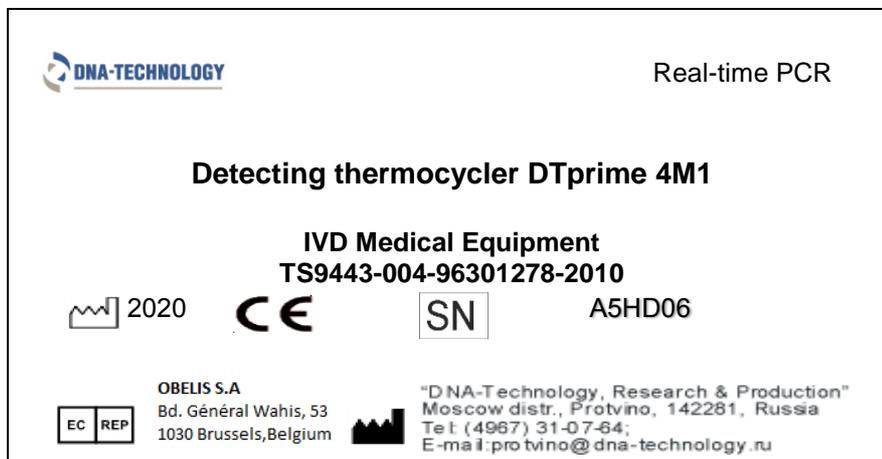


Fig.4. LCD panel

2.4. Marking. Symbols used for marking.

labeling of the instrument meets the requirements of the following standards: EN ISO 18113-1:2011, EN ISO 18113-3:2011, EN 61010-1:2010, EN 61010-2-101:2018.

Below provided example of the label on the rear panel of the instrument:



The following warning signs “Fragile”, “Top”, “Keep dry”, “Stacking is forbidden” are placed on the shipping box as well as:

- trade mark or information about manufacturer;
- brand name;
- date of production;
- net weight;
- gross weight;
- requirements for transportation and storage.

Symbols used for marking:

	<p>For in vitro diagnosis only</p>
	<p>Storage requirements (temperature)</p>
	<p>« Fragile »</p>
	<p>«Top»</p>
	<p>« Keep dry »</p>
	<p>« Stacking is forbidden »</p>
	<p>Date of production</p>
	<p>Manufacturer details</p>
	<p>High voltage</p>

3. Setting up the instrument

3.1. Instrument Unpack

DTprime real time PCR instrument is delivered packaged in a cardboard or wooden box with inserts of polyethylene foam; the package is intended to protect the instrument from mechanical damage when shipped or stored.

Before being packaged in the box, the instrument is placed to a special bag of durable and waterproof material with zip fastener and transportation straps.



Caution! Heavy! The instrument has to be carried to the installation's place in the manufacturer's package box by two persons.

Before taking the instrument out of the package box, perform the following procedures:

- provide a vacant space on a work table for the instrument;
- place the box with the instrument beside the table and open it;
- take the components out of the box, and remove the polyethylene foam inserts;
- compare the components with the delivery set (refer to item 1.2);
- observing all the precautions, lift the instrument by two persons using transportation straps and place it on a table;
- remove the bag and inspect the instrument visually for external damages.

If any items are missing or damaged, contact your local “DNA-Technology” distributor or “DNA-Technology” directly.

3.1.1. Instrument Carrying

During operation, the instrument may be carried over a short distance within the facility in unpacked condition by two persons or using movable table (trolley) to other preliminary prepared working place in accordance with corresponding precautions.

Note! In case the instrument is transported over long distances or shipped by a carrier, it has to be placed into the original manufacturer's package.

Caution! When instrument is moved the thermal block has to be in closed position. Otherwise, in case of some problem with instrument please contact service department of local distributor or “DNA-Technology” directly.

3.2. Instrument installation

Caution! The instrument has precision mechanical elements. To avoid displacement of them any shocks during operation and movement of the instrument should be eliminated.

When selecting a place for the instrument's installation, leave about 18 cm of space in the front and 12 cm in the all others sides of the instrument for sufficient ventilation and free access to the thermal block and power switch.

Plug the power cord into the back of the instrument and into an appropriate electrical outlet. Plug the USB 2.0 cable into the back of the instrument and into an appropriate PC USB port. Press the power switch on the back panel of the thermal cycler to start the system. For a complete power off the instrument must be disconnected from the power outlet and PC.

The instrument does not require any additional devices to stabilize circuit voltage. When plugging up the instrument to the devices of uninterruptable power supply, please note that provided power must be not lower than 550W.

Prior to connecting the instrument to the supply voltage, check the presence of protective grounding in the socket to which the instrument will be hooked up as well as integrity of the connecting cable.

Caution! Hooking up to the socket without grounding is prohibited.

It is important that the instrument be correctly grounded. The instrument is supplied with power cord that provides the instrument grounding, when connected to an appropriate power outlet.

Also it is important that the instrument and PC must be connected to the same grounded power outlet. Otherwise, there is a risk of the instrument damage.

Caution! After transport or storage under humid and cold conditions, dry out the instrument (2-3 hrs) at indoor temperature (+18-25°C) before connecting it to the supply voltage. During drying out the intrinsic protection may be impaired.

3.3. Software Installation

To control the instrument, software from the instrument's delivery set should be installed on PC. The software operates in Windows 98, Windows ME, Windows 2k/XP, Windows Vista, Windows 7, Windows 8, Windows 10.

Software installation is required only if the instrument and PC are purchased separately. If the computer is supplied as a part of the instrument set, the software has been already installed.

Caution! In case of purchasing the instrument without PC, any Windows operating system is not provided.

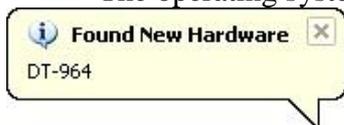
Software installation procedure consists of two stages:

1. Instrument driver installation.
2. Installation of the software.

3.3.1. Instrument Driver Installation

Driver installation for the instrument is identical with one for any standard device and should be performed as follows:

- Switch on PC and wait until the operating system is launched.
- Insert the USB flash drive with the drivers into the USB port of PC or download drivers from DNA-technology official website at <http://www.dna-technology.ru/eng/support/>
- Turn on the instrument using the power switch on the back panel.
- The operating system identifies a new device in 1-2 minutes (wait for message



on the computer screen) and start driver installation process using the Found New Hardware Wizard (Fig. 5)



Caution! When installing the driver on the PC with Windows 7, Windows 8 or Windows 10 operating system, the **Found New Hardware Wizard** window does not appear automatically. Do the following to install the driver:

- After connecting the instrument to the computer, open **Device Manager** window, then right-click **DT-964** and select **Update driver** in the list
- In the next window **Driver Updating** select **Manual driver search and installation**, click **Browse**, select **Drivers_DT96x_W32_2014** folder (for 32-bit version) or **Drivers_DT96x_W64_2014** folder (for 64-bit version).
- Click **Next** and complete the driver installation as described below.



Fig.5. Launching the Wizard for driver installation

- Select point **No, not this time** and click 

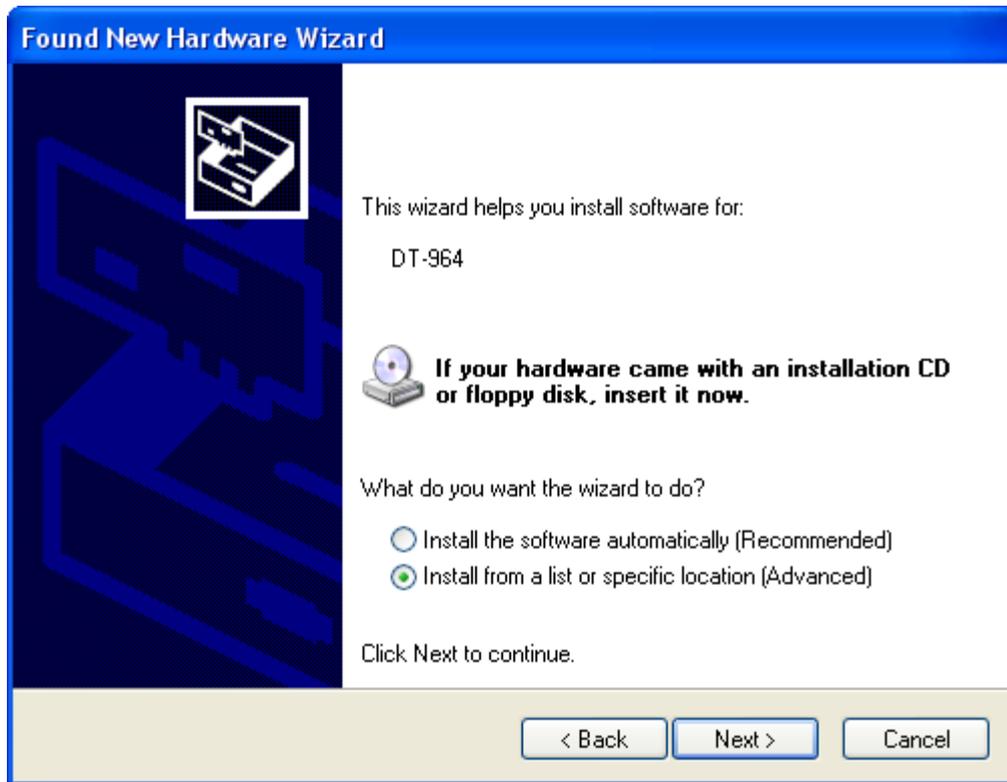


Fig.6. Selecting a type of installation

- Select **Install from a list of specific location** and click  (Fig. 6)

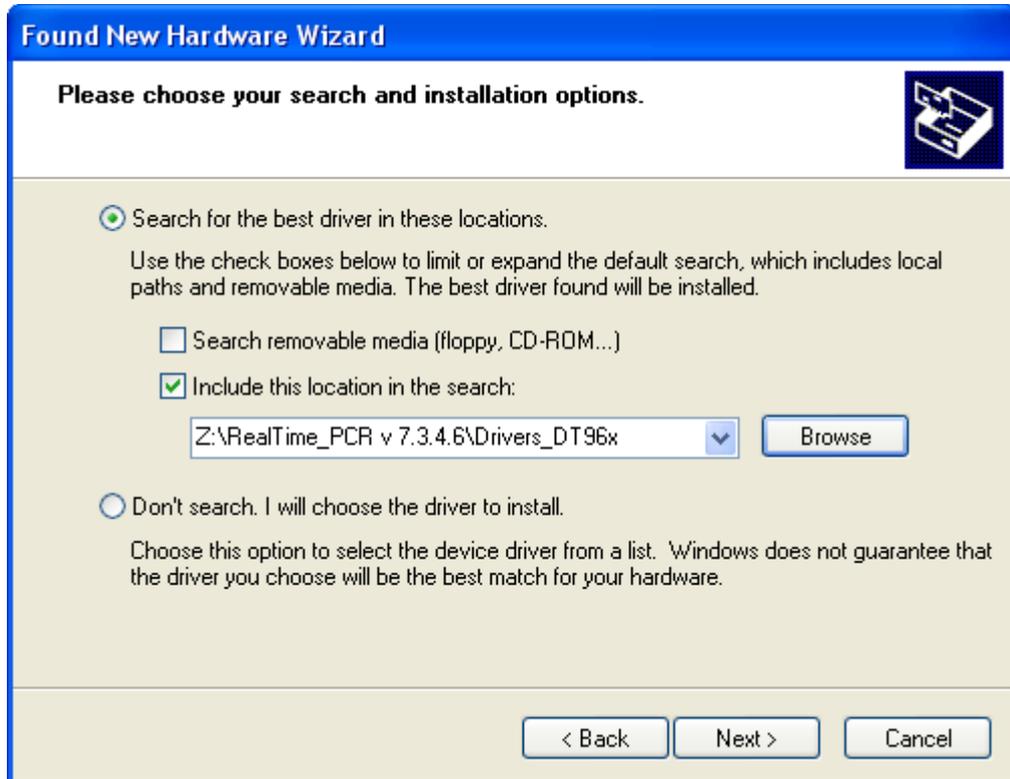


Fig. 7. Searching Drivers_DT96x folder on the USB flash drive

- Select a path to the driver folder by ticking **Add this location to the search** and click  (Fig.7).



Fig. 8. Selecting the Drivers_DT96x folder on the USB flash drive

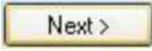
- In the pop-up window Browse For Folder select Drivers_DT96x folder on the USB flash drive and click  (Fig.8).
- Making sure the driver folder path is correct click  (Fig.9),



Fig. 9. Selecting inf-file on the USB flash drive

Select the latest version of the driver and ignoring message **The driver has no digital signature** confirm the driver selection by clicking  (Fig.9)



Fig.10. Further Driver_DT96x installation

- confirm the driver selection by clicking **Continue Anyway** (Fig. 10).
- Select the path to Dt964.sys file on the CD by clicking **Browse** (Fig. 11).

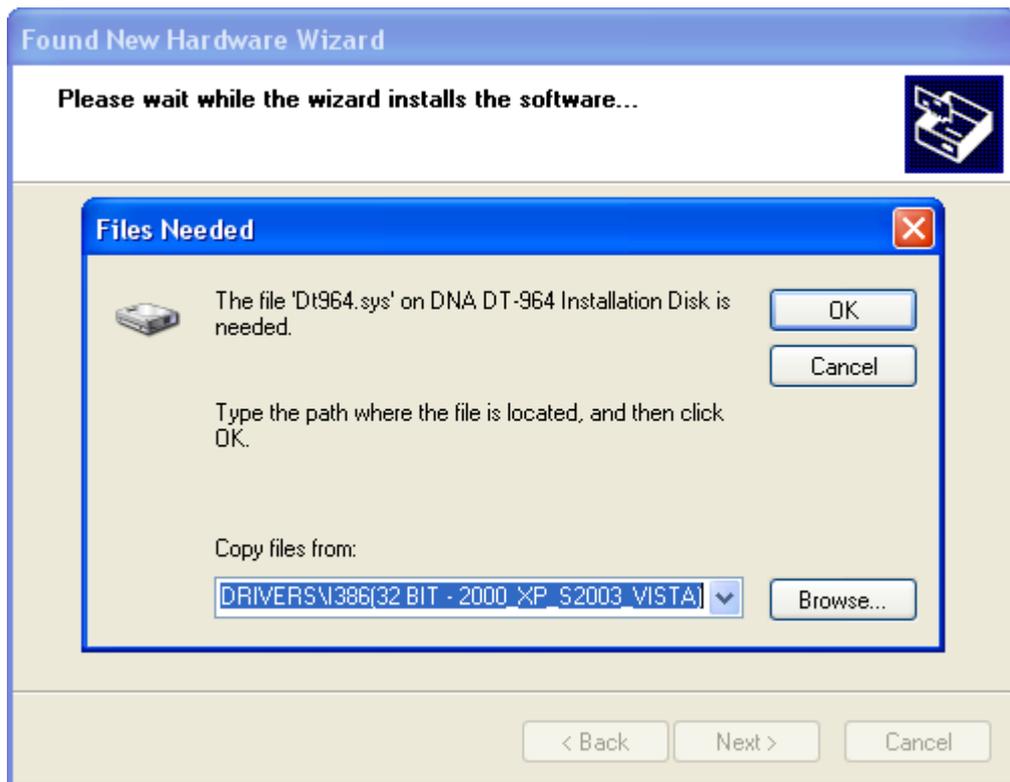


Fig.11. Selecting the path to Dt964R.sys file on the CD

- In the next window **Locate file** find this file in the Drivers DT96x folder on the CD, select it and click **Open** (Fig.12)

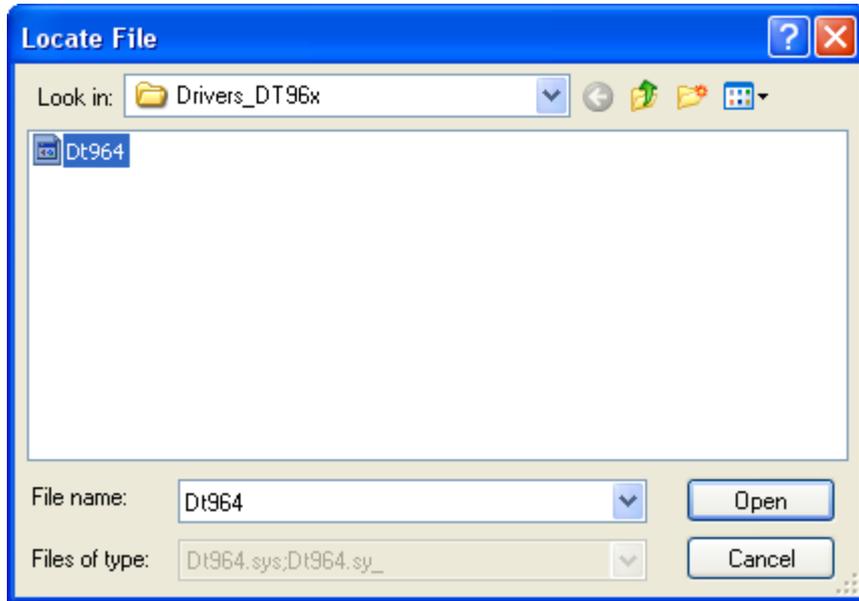
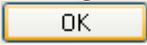


Fig.12. Confirmation of Dt964R.sys file selection on the USB flash drive

- Making sure that the path to the file in the window (Fig.11) is correct, click .
- After message that the installation of the driver has been completed (Fig.13), click .

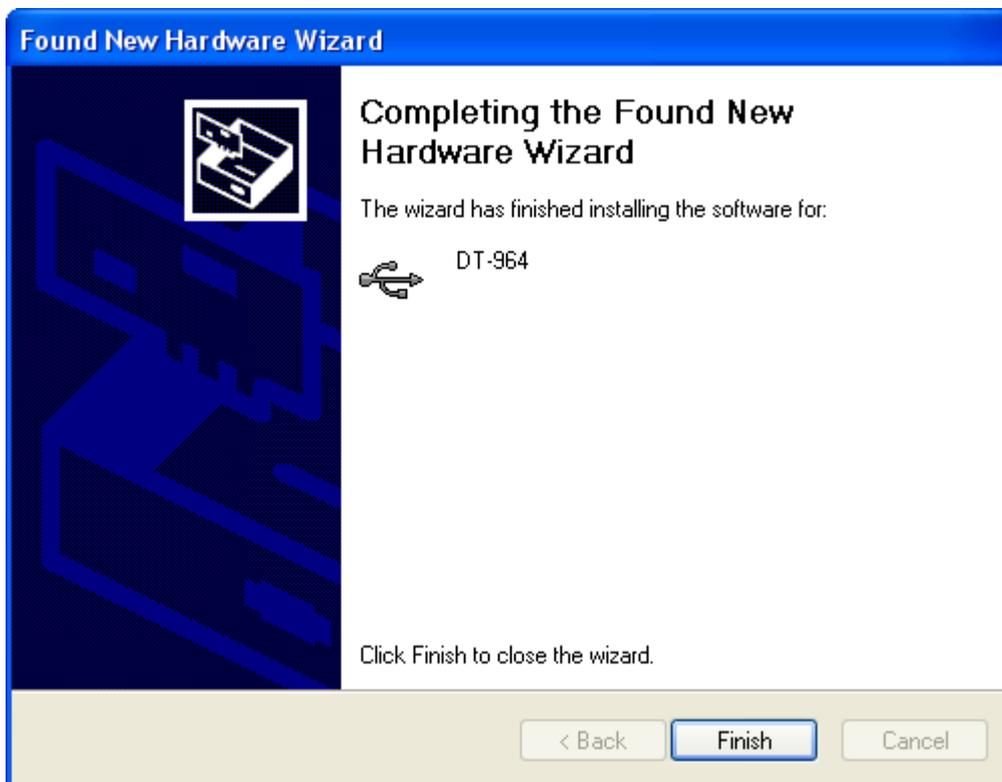


Fig.13. Driver installation completion

If driver installation is successfully completed, the system will display a message



Note! Installation of the instrument's driver is required upon the first connection only.

3.3.2. Installation of RealTime_PCR software

Note! The software must be installed on the computer by a user with administrative privileges.

Make sure you are logged in with administrative privileges.

Installation of RealTime_PCR software should be performed as follows:

- Launch Setup manager from the USB flash drive included in the delivery set (it can be also downloaded official website <http://www.dna-technology.ru/eng/support/>).
- Select Russian/English language to use during the installation (Fig. 14) and click

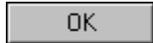


Fig.14. Selecting a language

- Setup Wizard appears with the version of software (Fig.15).



Fig. 15. RealTime_PCR installation Wizard

- Select a folder for software installation (Fig. 16). **RealTime_PCR** software can be installed into a default folder or any folder on the hard disk by clicking  (installation into a default folder is preferable).

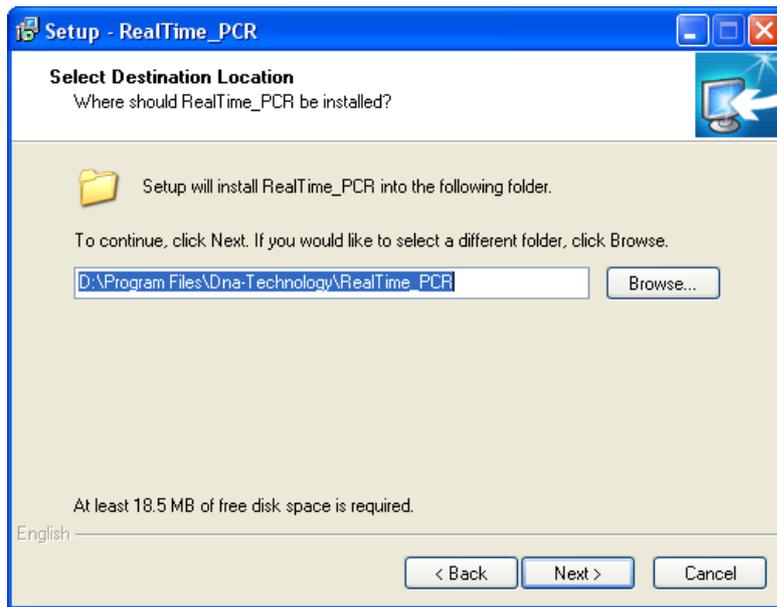
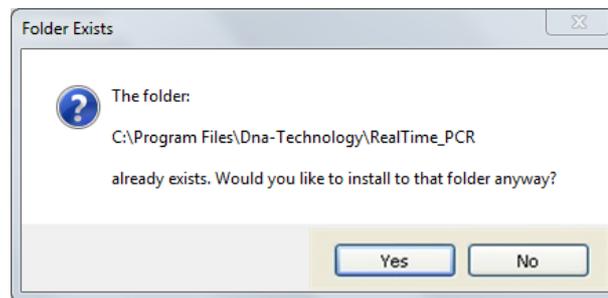
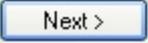


Fig. 16. Folder selection to install software

- Having selected the installation folder, click .



- Select the name of the folder, where **RealTime_PCR** shortcuts will be created, and click . (Fig. 17).

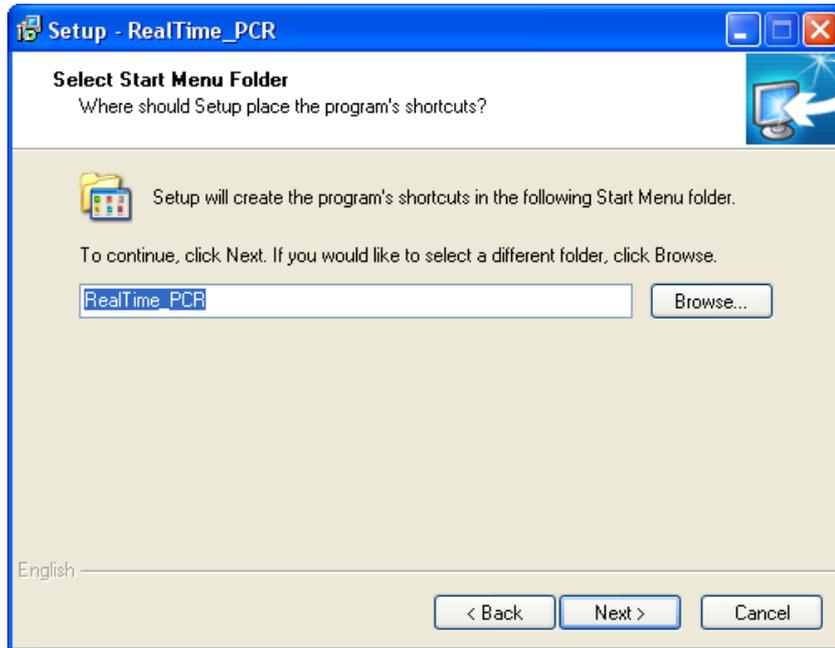


Fig. 17. Selecting of folder to create shortcuts

- Confirmation window will appear, informing that software is ready for setup (Fig. 18). Check the chosen options and click  .

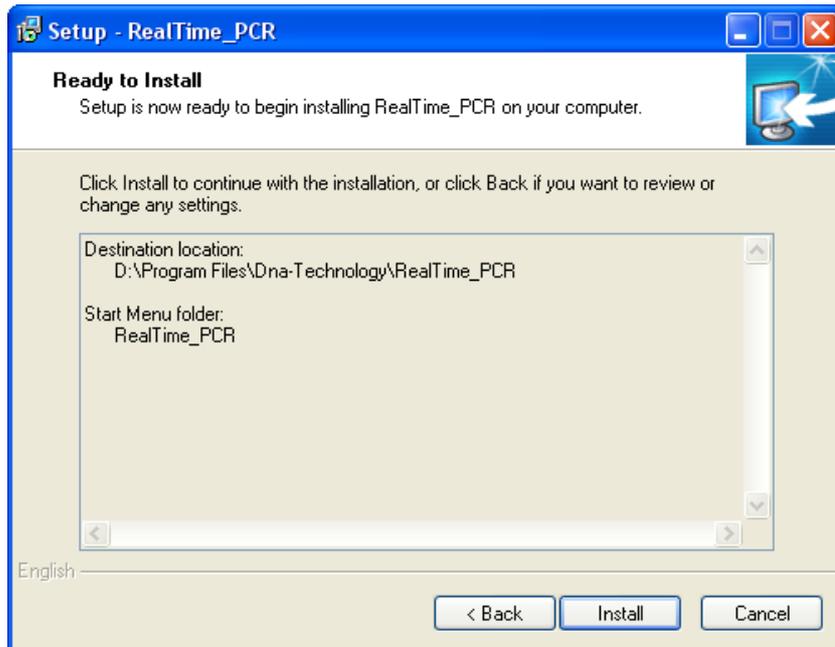


Fig.18. Confirmation window

- After the installation is completed you will see following window (Fig. 19). Click  to quit the Setup Wizard.

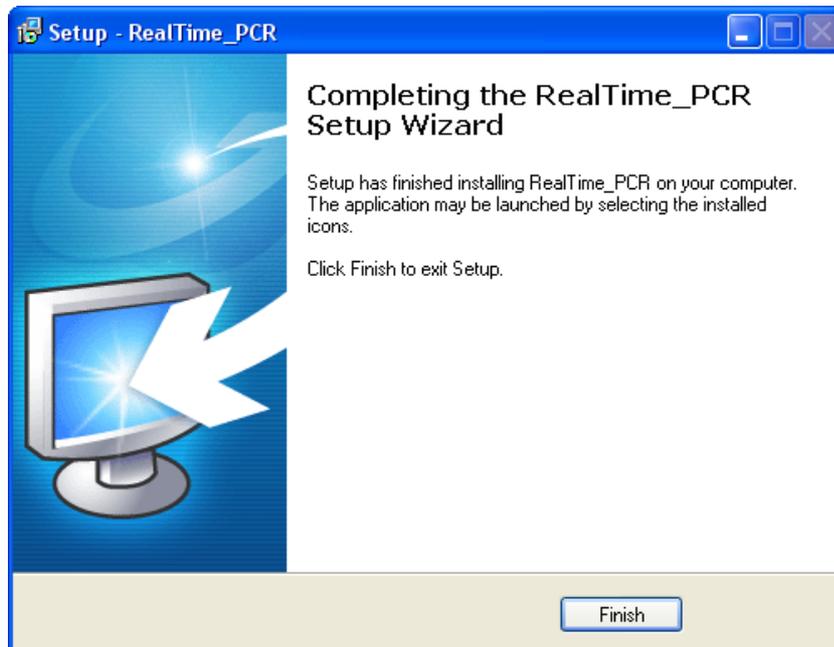


Fig.19. Completed operation of the RealTime_PCR installation wizard

Caution! To ensure multi-instrument operational conditions, the corresponding **Server_RTDevice** service is included in the **RealTime_PCR** software. This service is started at the first start of the software in the mode Device handling with sign  displayed in the **tray** and stays active until the computer shutdown.

By right-clicking on this sign, the following box can be opened:



Choosing the **Show List_Device** shows the list of instruments connected to the PC.

1	A5Z401	96	0x1964	0x199A	0x00000000
2	A5Z405	96	0x1964	0x199A	0x00000000
3	A7Z401	48	0x1964	0x199A	0x00000000

Clicking the **Close Server_RT** stops this service at the PC.

4. Operating Procedure

4.1. Instrument Switching-on and RealTime_PCR Launch

Check whether the instrument is connected to the PC via USB cable, and then switch the instrument and the PC on.

Start the RealTime_PCR software using  icon on the **Desktop** or via the **Start menu** of the Windows. In the window of selecting operation modes (Fig. 20) **Guest** operator is a default (not recommended to use). Select another operator by using drop-down menu or add a new one (drop-down menu last line). Please type in the name of **Operator** and select the **Directory** where run protocol files and assay results be stored. Click Ok and select **Device operation**.

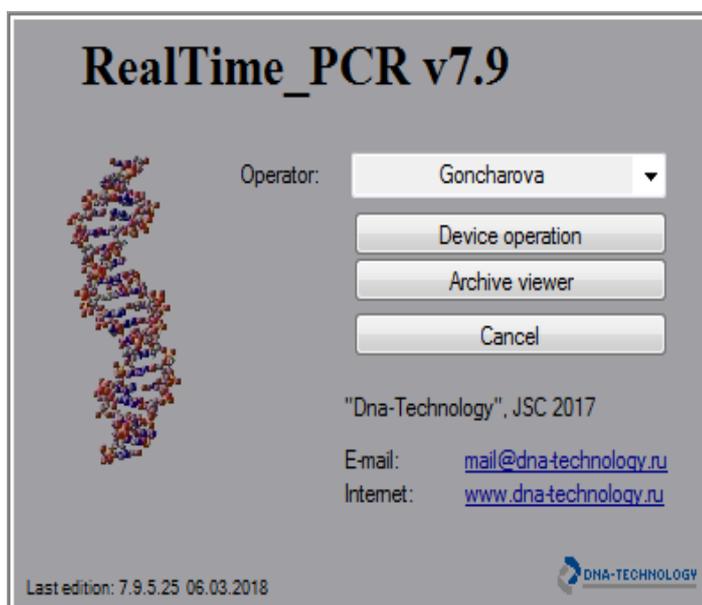


Fig. 20. RealTime_PCR start window

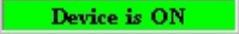
Once **RealTime_PCR is launched** in the **Device operation** mode a window of running program with an indication of the instrument status is displayed. In the status line **Device is OFF**, and the dialog window **List of devices** available instruments are listed (Fig. 21).



Fig.21. Window of connected devices

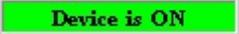
Select the instrument to start and click .

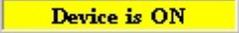
The instrument will be connected to the PC and the indication in the status line of the operation procedure window will be changed to  .

After instrument self testing, indication of its status in the status line will be changed to  and the instrument will be ready for running the amplification program.

A detailed procedure of starting and operating RealTime_PCR software is described in the second part of the operation manual named **Software guidance**.

Indication of instrument status in the status line means:

 – the instrument is on and ready for operation (green background).

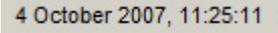
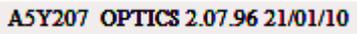
 – the instrument is on, however, is not ready for operation (yellow background) – the warm-up is in progress. After warming-up (which is normally not longer than 10 minutes since switch-on), the background color will change from yellow to green.

 – the instrument is either switched off at the moment of launching software, or is not connected to the PC via USB2.0 A-B cable or is not selected in the window **List of devices**.

Note! Several instruments can be controlled by one computer. In this case the instruments have to be connected to the computer via USB ports.

When working in a single-device mode, the operator connects and disconnects a selected instrument by clicking  and  in the **List of devices** window, which is displayed automatically at the launch of RealTime_PCR, and while operating this window can be opened by clicking **Preferences/ List of devices** at the menu bar.

When working in a multi-device mode, each instrument requires launching of its RealTime_PCR software in an individual window. In this case operation of several instruments may be controlled simultaneously.

The status line represents current date and time  , instrument serial number and a version of optical controller firmware  .

4.2. Preparing the Instrument for PCR

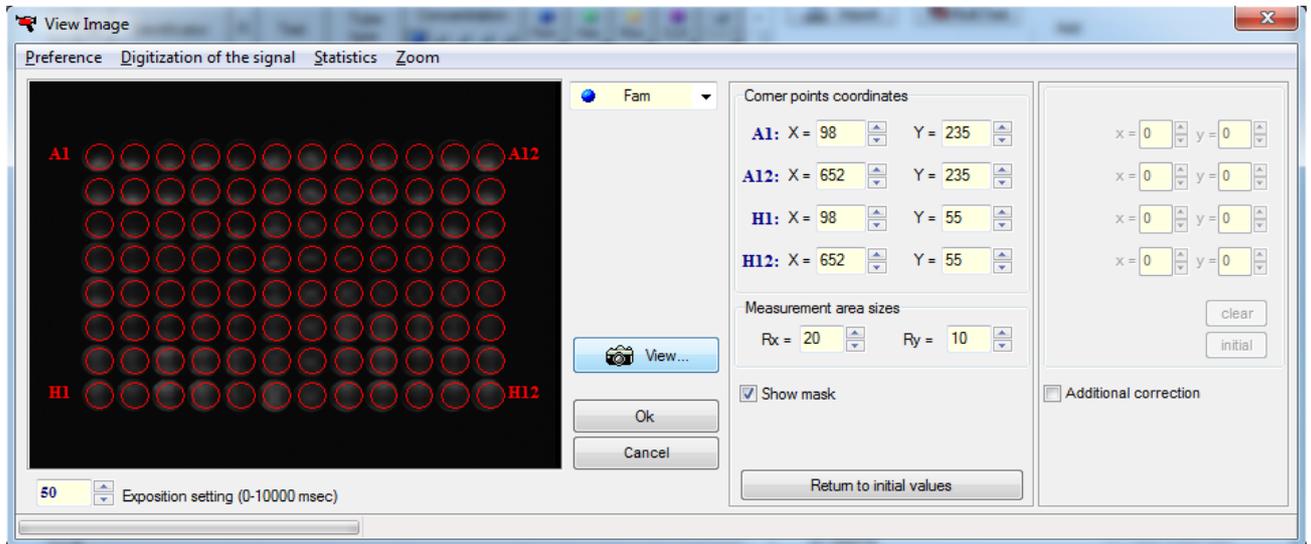
The instrument readiness to run the PCR assay is checked automatically every time it is switched on. While this radiator temperature, thermal plate temperature, and the hot lid temperature are checked against permissible values.

4.2.1. Checkup of Geometrical Settings

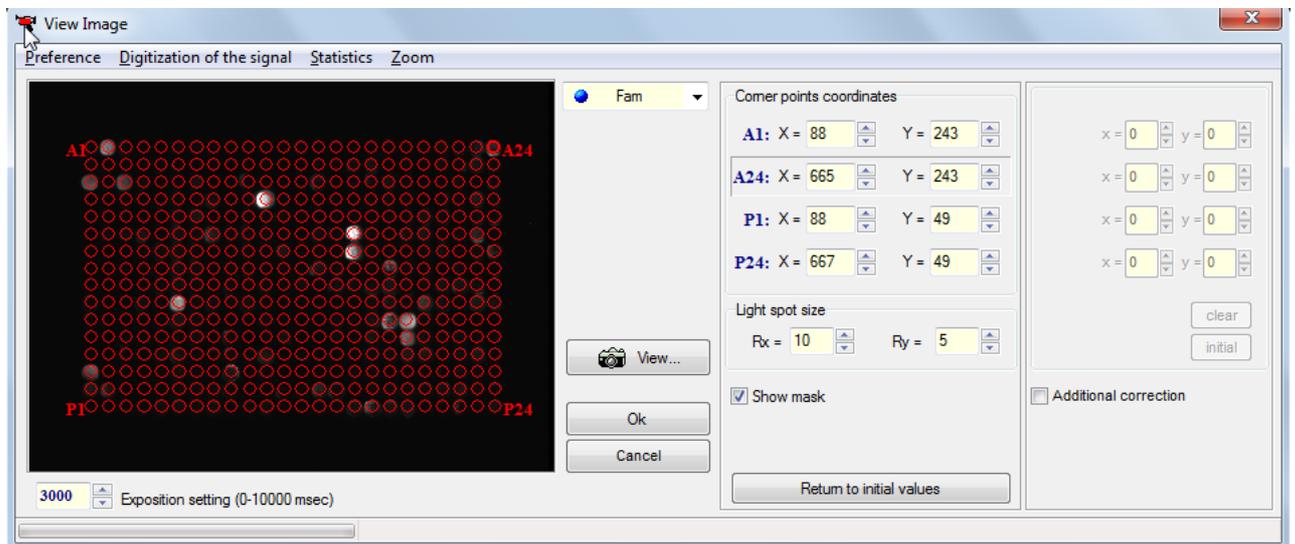
Attention! At the first switch on of the instrument after its transportation or any other change of its position, checking the optic module's geometrical settings is strongly recommended.

A detailed procedure of the geometrical settings checkup is described in the second part of the operation manual named **Software guidance**.

The image of thermal plate wells outlining the boundaries of a measuring region (red circles) has to completely encompass the light spots. If the circles are displaced relative to the light spots, correction of the geometry of an optical image must be carried out (Fig. 22).



a)



b)

Fig.22. Check of geometrical settings (a – M modification, b – X modification)

4.2.2. Optical Measurement Exposure Setting

Preset working exposure values for each channel are the following:

Fam – 1000; Hex – 1000; Rox – 1000; Cy5 – 1000; Cy5.5 – 1000

Note! Preset parameters of optical measurements for each channel are optimal to operate with DNA-Technology PCR kits and could be suitable for the most of third party kits.

Exposure change is required only when levels of different channels' optical signals substantially differ, as it may result in incorrect color compensation in the process of analysis of optical measurements. For exposure setting window see Fig. 23.

Caution! In case of using third party kits the optimal exposition values for channels must be provided by manufacturer.

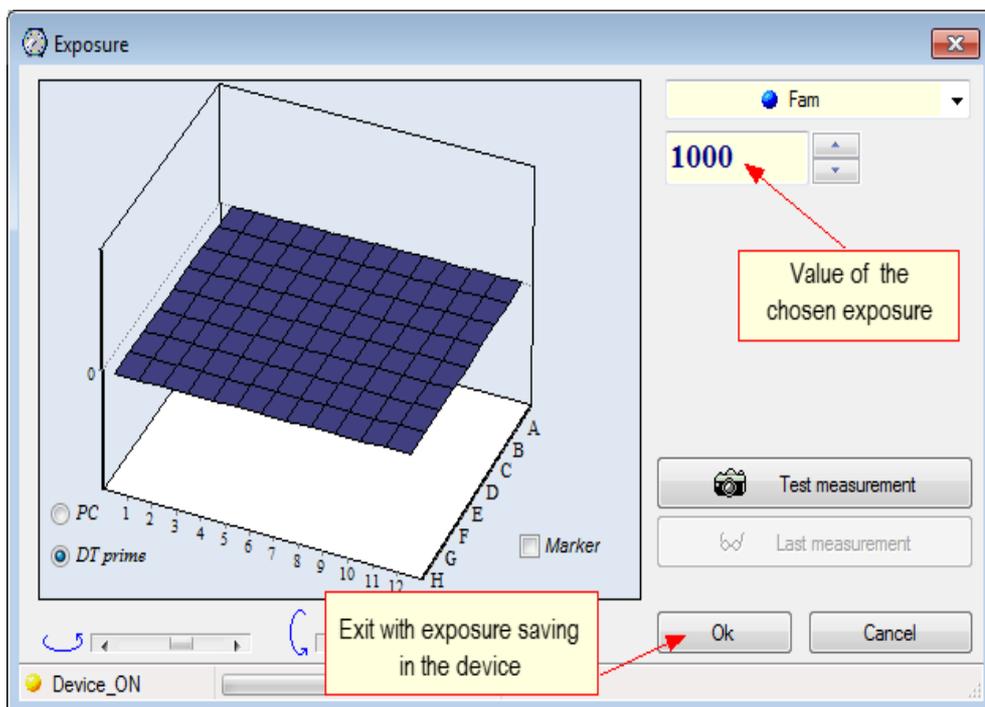


Fig.23. Exposure setting window

A detailed procedure of exposure value selecting is described in the second part of the operation manual named **Software guidance**.

4.2.3. Checking the Cleanness of Thermal Plate Wells

While checking the optical unit geometrical settings, one has to increase the value of exposure in 2 times in comparison with the operational values. There must be no bright spots on the image of empty thermal plate wells.

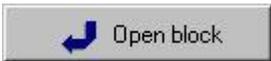
It is recommended to check the purity of the wells before placing tubes, strips or plates with PCR mixes, if there was a risk of contamination before.

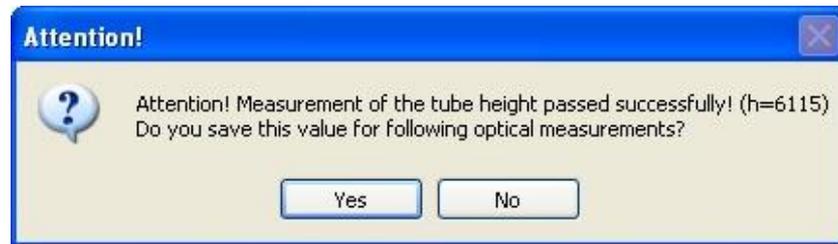
Note! Routine check of well cleanness must be carried out by the operator in compliance with the instrument maintenance procedure (see item 5).

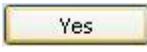
4.2.4. Tubes Height Measurement

Attention! The height of the test tubes must be measured every time new type or brand of plastic consumables is used, to ensure proper pressure of the hot lid.

To perform the tubes height measurement procedure:

- Click  and then place tubes or stripes uniformly along the thermal plate;
- Select Preference/Device diagnostics/Measure height of the tubes in the menu bar and wait for the message:



- Save measured value by clicking  .

Caution! To measure tubes height, use at least 32 tubes or 4 strips putting them evenly throughout thermal plate.

4.3 Creation of Plate protocol and Amplification Program

After switching on the instrument create or edit of a plate protocol and amplification program using RealTime_PCR software.

4.3.1. Filling in of Plate protocol

The window for plate protocol filling in (Fig. 24) is displayed immediately after starting RealTime_PCR and comprises: data input table, control buttons for plate protocol filling in and a field of graphic representation of tubes arrangement in the thermal block.

Fill-in plate protocol in one of the following ways:

- by using **Test** procedure with saved standard assay parameters (recommended for standard assays in clinical laboratories);
- by using plate protocol templates saved before.

Note! Do not fill in the plate protocol without using Test procedure, as window Analysis Parameters won't be active, and the optical calibration coefficients of the instrument will not be used.

In the course of the filling in the plate protocol, enter the following data:

- identification code (description) of each tube;
- type of each tube (sample, standard or control +/-);
- the number of replicas of each sample;
- concentration of calibration samples (in a quantitative assay);
- fluorophore is used (fluorescent label) and its purpose (specific target, IC or not detected);
- arrangement of tubes in the thermocycler block;
- Plate protocol No.;
- operator's name.

The procedure of creating (editing) the plate protocol is described in details in the second part of the operation manual **Software guidance**.

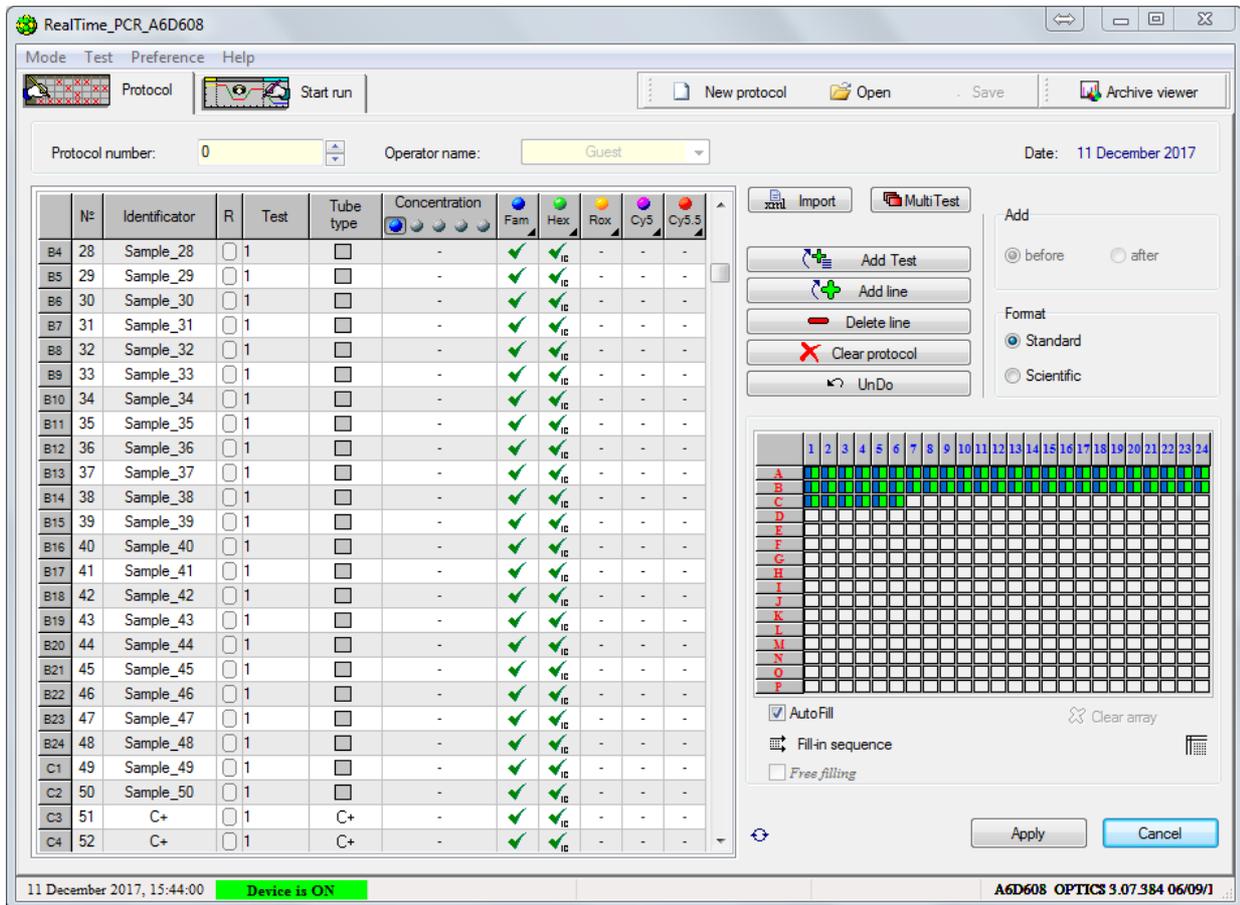
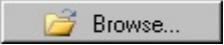
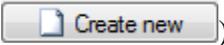


Fig.24. Window of plate protocol filling-in

4.3.2. Creation/editing of Amplification Program

After filling in the plate protocol proceed to the **Start run** tab. The amplification program, specified in the **Test** procedure used for plate protocol filling in, will be displayed as the default. If the plate protocol was filled in without using the Test procedure, the amplification program of the last run will be displayed.

If required, the following steps may be carried out:

1. Open the amplification program by clicking .
2. Create a new amplification program (click ) by using the Amplification program editor or choosing amplification program template (Fig. 25).
3. Edit the existing amplification program by clicking  (Fig. 26).

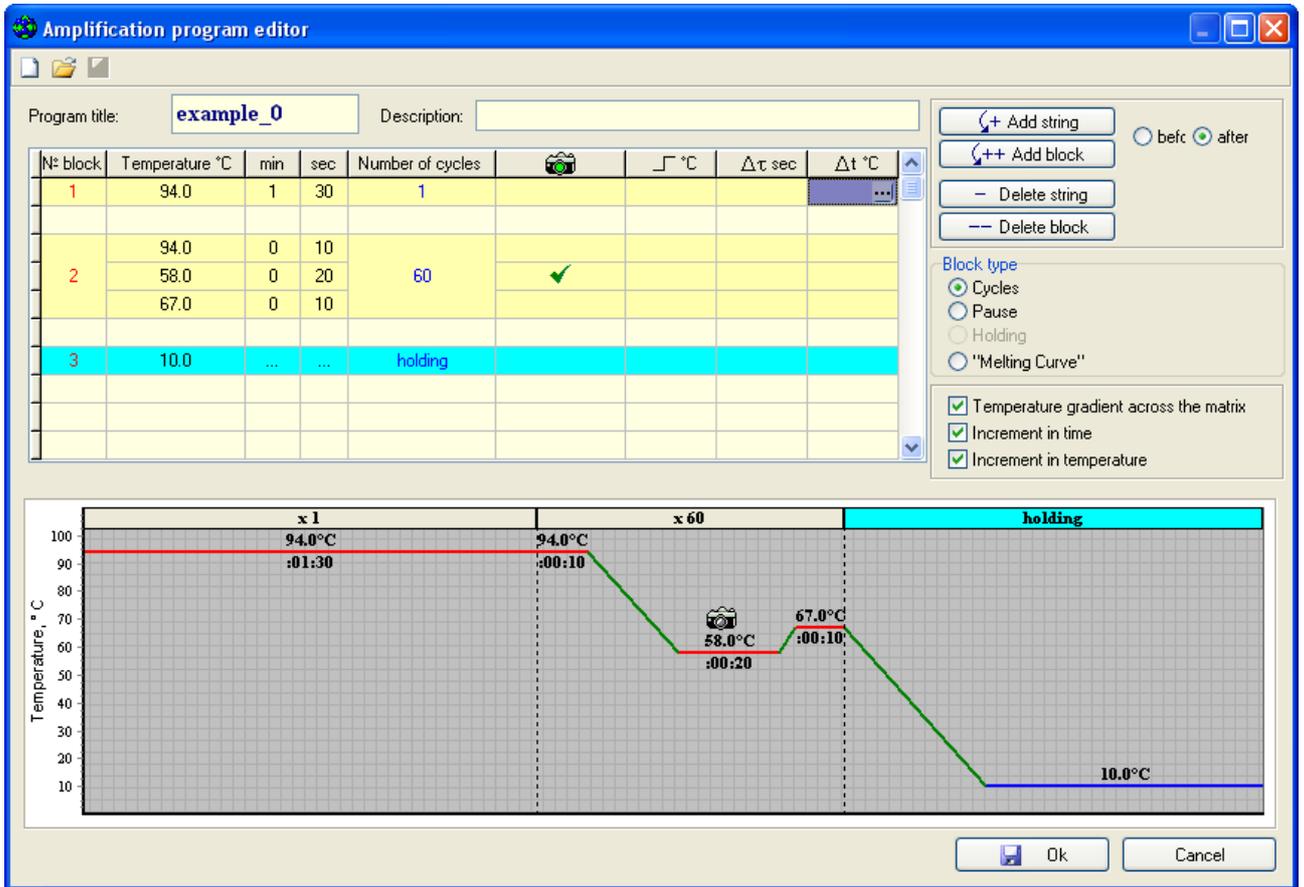


Fig.25. Creation of amplification program using template

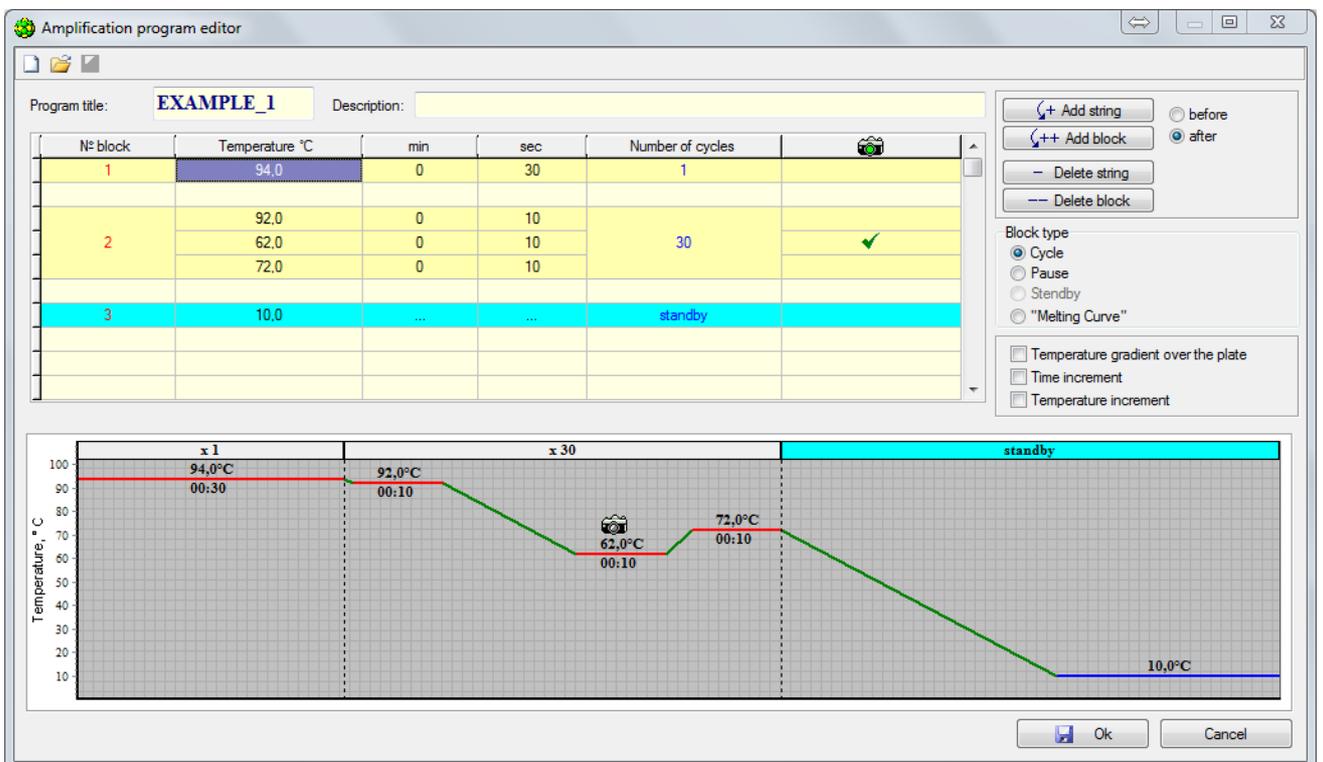


Fig.26. Edit of existing amplification program

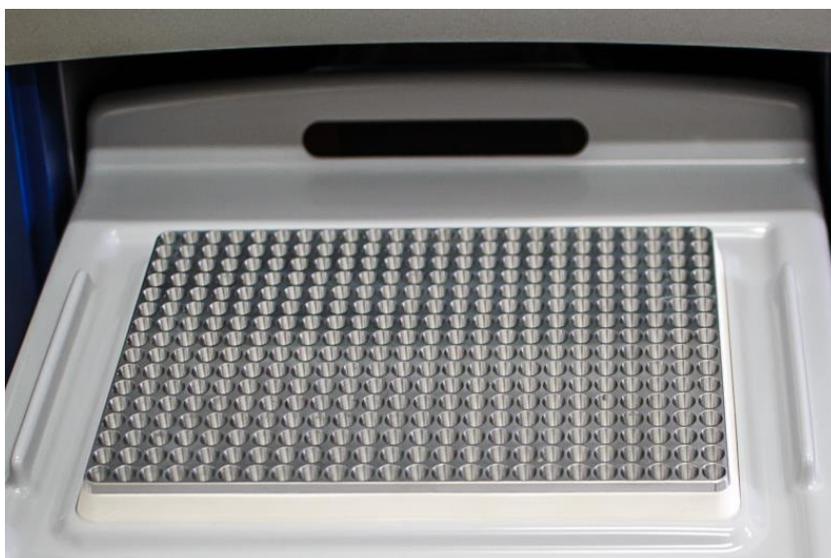
Creating and editing the amplification program are described in details in the second part of operation manual **Software guidance**.

4.4. Placement of Tubes with Samples

To place or remove the test tubes with samples, do the following:

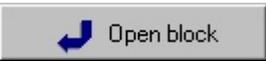


a)



b)

Fig.27. Exterior appearance of the thermal block in open position (a – M modification, b – X modification)

1. Click  in the **Start run** tab or push the button of thermal block manual control and wait until the thermal block is opened (Fig.27).

2. If it is the first start of the instrument, rub the wells of the thermal block with a cloth moistened in alcohol (96-% ethanol or 100-% isopropyl alcohol may be used) as described in section 8 of instrument maintenance. In the course of operation, wipe the wells as set forth in the Maintenance section.

3. Place the tubes with samples into the thermal block wells in compliance with filled in plate protocol.

4. Click  in **Start run** window or press the button of instrument manual control and wait until the thermal block is closed.

Caution! Be sure to place at least 16 tubes in order to prevent their damage because of hot lid pressure. If there are less of 16 tubes please add empty tubes of the same height to make them as it is required.



Caution, dangerous! The instrument provides automatic loading and unloading of tubes from the thermal block in the operational position. The command to move (open or close) of the thermal block comes from the operator. It is forbidden to carry out any manipulations during the movement of the thermal block because it can lead to damage of the movement mechanisms of it. If an obstacle appears in the process of the thermal block closing, it automatically switches to the opening mode. To prevent the failure of the movement mechanism in case of a mechanical obstacle occurs during the movement of the thermal block, the front panel of it is made flexible.

Caution, hot surface! During the instrument operation, the matrix of thermal block and hot lid may get hot up to 100 and 105 °C respectively. When execution of the PCR RUN (operating mode) the thermal block matrix and the hot lid are not available for operator. Upon completion of the PCR RUN operator is not allowed to remove the tubes from the thermo block until the instrument enters in storage mode (low temperature mode) with the following message:



4.5. Startup of the amplification program

Enter necessary notes such as the particularities and characteristics of the upcoming run and the PCR mix volume in tubes;

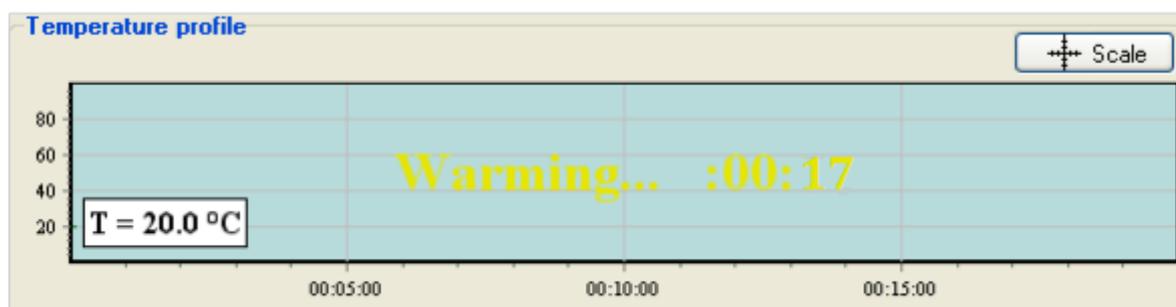
Be sure that the instrument is on and properly connected to the computer, which is confirmed by yellow **Device is ON** or green **Device is ON** indicator in the software status line.



Start the amplification program by clicking

When running the amplification program, select a file name, data format and folder to save future results of the upcoming RUN.

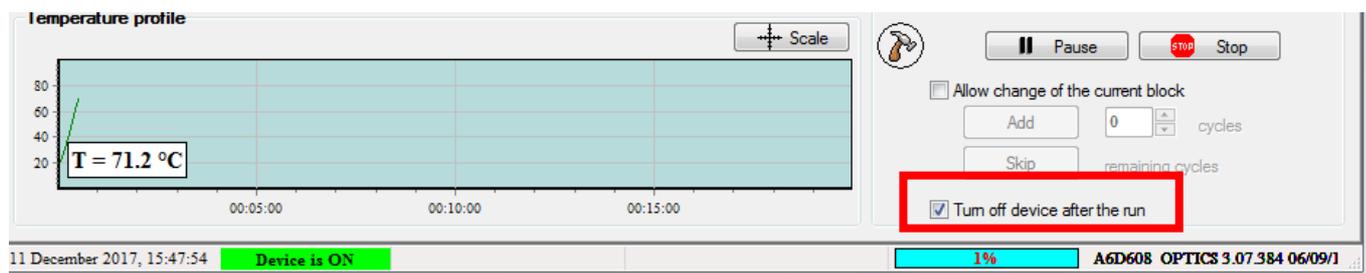
Note! If **Run** is started while the instrument is in the process of warming up (yellow indicator **Device is ON** in the software status line), blinking message **Warming** will be appeared in **Temperature Profile** window:



The same message will appear at the instrument's display as well. When warming up is completed, the instrument will automatically proceed to the RUN execution mode.

In case of necessity of instrument's autonomous functioning after the RUN completion and its automatic turning to sleep mode perform the following:

1. In **Running** window activate the option - turn off device after the RUN



2. Close the Real Time PCR software by clicking  in the upper right corner of its window.

3. When RUN is completed the instrument will switch to the sleep mode, what is confirmed by blinking diode on the front panel of the instrument.

Note! The instrument will proceed to the sleep mode only if “**Turn off device after the run**” option is activated and the executed amplification program finishes as **Holding** mode.

4.6 Amplification program running

Running of the amplification program is controlled via RealTime_PCR software.

The following options could be done during its execution:

- pause running;
- stop running;
- add any number of cycles in a current block or skip execution of the remaining cycles and proceed to the execution of the next temperature block;
- edit the log of optical measurement;
- to check optical measurement data of the selected tubes in the form of 3D histogram in the **Distribution of optical measurements over the plate** window by clicking  (Fig. 28).

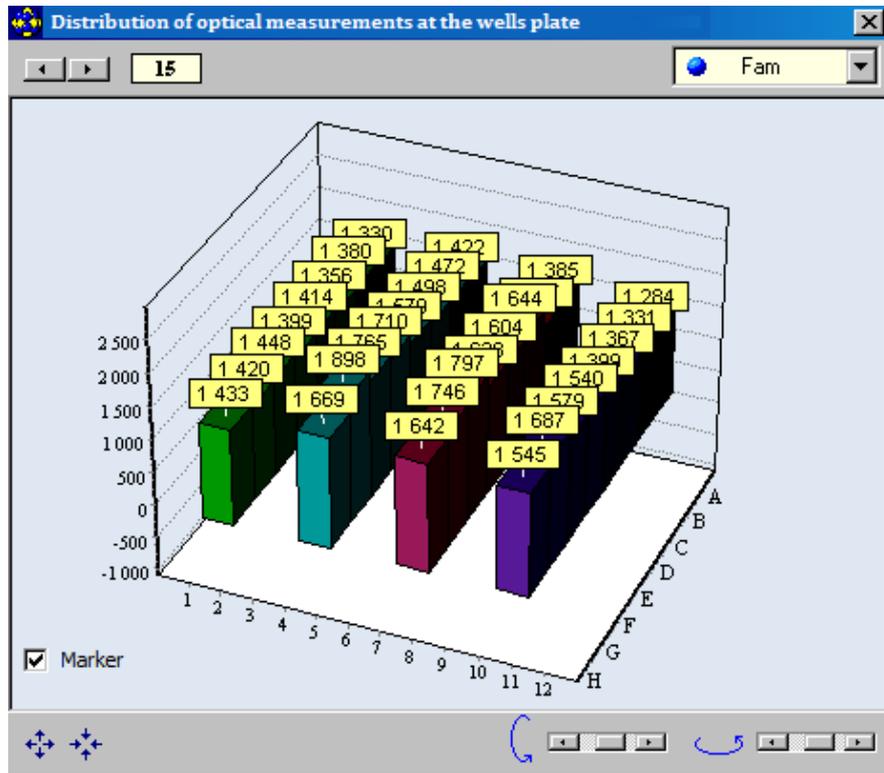


Fig.28. Distribution of optical measurements over the plate window

If option “Turn off device after the run” was not activated, the instrument passes to the **Holding** mode after completion of the RUN (Fig. 29).

In this case, a blinking message Holding... and a information about storage temperature will appear on the chart.



Fig.29. Message about the RUN completion

The same message will appear on the instrument display as well.

When the temperature reaches the value close to holding temperature information message appears on the screen notifying that the RUN has completed and instrument passes to the holding mode (Fig. 30).

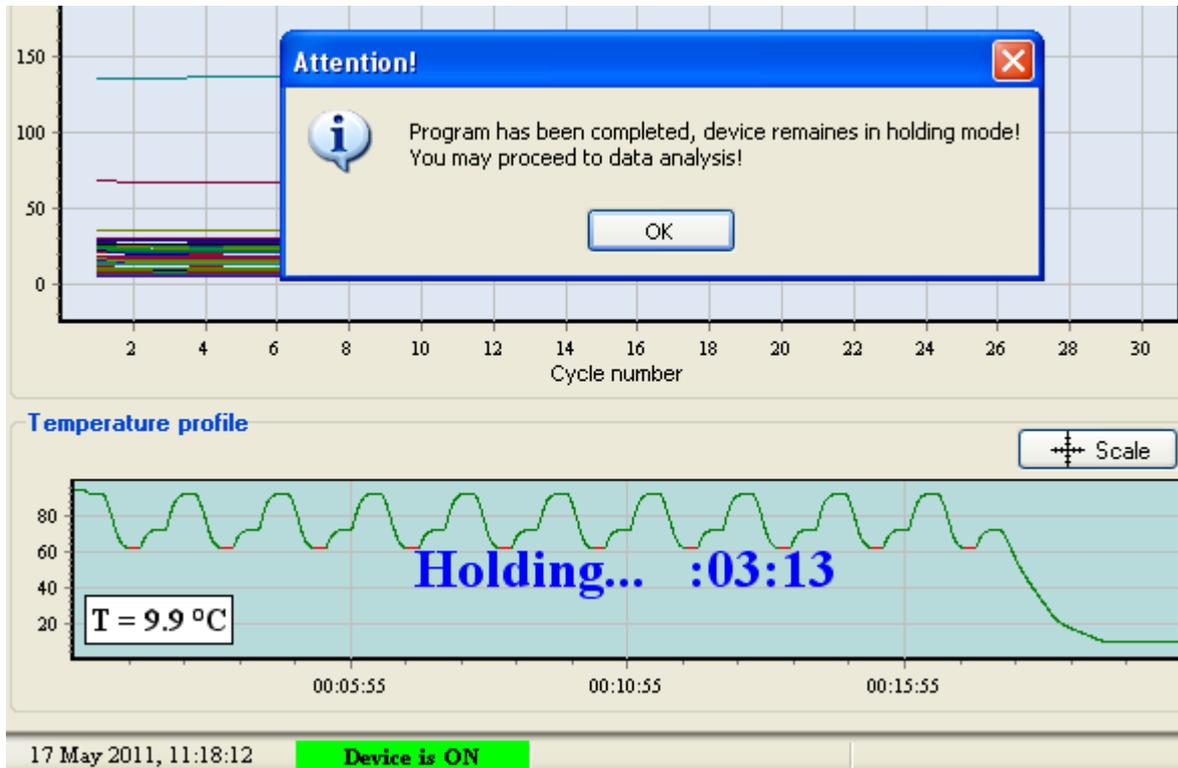


Fig.30. Storage mode indication

By pressing button **OK** **RealTime_PCR** software is switched to **Archive viewer** mode (**Data analysis** tab).

If option “**Turn off device after the run**” was activated, then upon completion of the RUN the instrument switched to the sleep mode. To exit this mode, launch **RealTime_PCR** software and again connect to the instrument in the appeared window **List of devices**.

Note! To read results of the executed RUN after exit sleep mode proceed to menu **Preferences/Last run in device** option.

The analysis of optical measurements results are described in details in the second part of operation manual **Software guidance**.

5. Maintenance

5.1 General Guidelines

The maintenance of the instrument is necessary to keep it at optimum working condition, which minimises the risk of having unscheduled downtime and providing for its maximum operational life.

The instrument belongs to IVD medical equipment. Maintenance should be carried out only by competent personnel, who have studied the present operation manual.

The instrument is required for minimal routine maintenance during normal laboratory operation.



Caution, risk of electrical shock! Replacement of fuses should be done on de-energized instrument only. Fuses type is T10AH250V with the following parameters - 10A, 250V (slow with high breaking capacity) and 5x20 mm size should be used in the case of

their replacement. De-energizing of the instrument is achieved by removing the power cord connector from it or from the power outlet and disconnection of USB cable as well.

The instrument should be protected from mechanical shocks and any liquids getting on its case.

To keep thermal block matrix and elements of the optical system clean, the instrument thermal block has to be always closed (except operations for placement / removal of PCR tubes with samples).

Caution! To avoid failure of the instrument's optical system it is strictly forbidden to use any substances (heat-resistant pastes, oils, etc.) to improve contact between PCR tubes and thermal block wells.

5.2 Maintenance Procedure

The following procedures should be carried out by qualified personnel with a periodicity specified below:

- 1) Visual inspection of the instrument to assure the absence of damages on its surface. The condition (integrity) of the power cord and its reliable connection to the instrument. Frequency of visual inspection: before each switch-on.
- 2) Timely removal of some dust / dirt from the instrument's surface by using disinfectant solutions in accordance with regulations settled in an institution. Frequency: as and when the instrument gets dirty.
- 3) The exterior and functional surfaces of the instrument should be periodically treated with disinfectant solutions to eliminate possible contamination of it by amplification products of nucleic acids in accordance to local regulations and rules. Cleaning of thermal block wells has to be done by a cotton swab wrapped on a wooden rod and moistened with no less than 70-% ethanol.

Caution! Never use any metal objects (paper-clips, wire, etc.) for wells cleaning!

Attention ! When cleaning the wells and thermo block matrix do not allow a liquid flowing inside of the thermal block and the instrument!

Cleaning frequency: after 20 executed RUNs image of thermal block matrix should be checked for all channels (as it is described in point 4.2 of the present manual). In case of some bright spots will be detected for any ranges of fluorescence registration, cleaning of the wells have to be done.

5.3 Disinfection requirements of the instrument prior to maintenance and repair procedures.

The user is responsible for the proper usage of the instrument as well as for its disinfection procedures before carrying out maintenance or repair operations.

Prior sending the instrument for repair or maintenance to local service office or manufacturer it is necessary to perform the disinfection operations with accordance to local rules and regulations and fill out the disinfection certificate (see Appendix 1).

5.4 Troubleshooting

Trouble description	Probable cause	Corrective measures
After instrument switch-on there is no information shown on the LCD display	No voltage in the mains	Check if voltage available, socket works properly
	Poor contact or breakage in the power cord	Check the contact of power cord. If necessary replace the cable with a similar one (10A, 250V, 3x0.75mm ²), change T10AH250V fuses (10A, 250V), which are included in the set of delivery. It is allowed changing the failed fuses only once!
Status line of RealTime_PCR software shows message "Device is OFF" while the instrument is switched on.	Poor contact or breakage in the communication cable with the PC	Check the USB cable between instrument and PC
	Instrument driver is not installed	Install the instrument driver (see item 3.3.1)
	Failure in the Windows system	Reset the PC
After 10 to 15-minute warm-up process of the instrument, the yellow background of "Device is ON" message in the status line of the software doesn't turn into green	The instrument is malfunctioned	Please contact local service office or service department of the manufacturer
During the amplification program execution the following error message is displayed: "Error! " Program is running, but device does not reply!	Connection between the PC and the instrument is failed	Restore the connection between the PC and the instrument. Replace USB cable with a similar one. RealTime_PCR software will connect to the instrument and read out all the data missed without amplification program interruption. If this message keeps showing, please, restart the application

6. Storage and shipment

Caution! In case of storage in the cold conditions the instrument has be kept indoor at temperature +18 +25 °C during at least 4 hours prior to switching it on.

The instrument should be stored indoor with natural ventilation at the temperature range from + 5°C to + 40°C and relative air humidity to 80% at 25°C. In case of long-term storage, when it is not used, the instrument should be kept in the manufacturer's package.

A room, where the instrument is stored or operated, should be free of dust, acid and alkali vapors, corrosive gases and other harmful substances that may cause corrosion of the metal parts and electric insulation breakdown.

During transportation, the instrument should be protected from dust and precipitation. **It is forbidden to tilt and turn over the instrument!** Prior to the shipment, the instrument should be secured to ensure its stable position and exclude any displacement and shocks.

Instrument can be shipped by any kinds of transport and it has to be carried in the manufacturer's transport package and treated with accordance to manipulation signs applied on the outer surface of transport package. Acceptable environmental conditions for transportation are the following: temperature from - 50°C to + 50°C and relative air humidity up to 80 % at + 6°C.

Note! The instrument contains precision mechanical parts. To avoid their damage during handling and shipping, the requirements of manipulation signs applied on package should be strictly followed.

Attention! The instrument has to be shipped with the closed thermal block only.

7. Recycling

7.1 Recycling of medical devices has to be carried out in accordance with the classification, rules for the collection, use, disinfection, storage, transportation, accounting and disposal of medical waste established by the authorized federal executive authority.

7.2 Recycling of the instrument has to be carried out by special companies, which have the appropriate license, using specially equipped places in accordance with the requirements stipulated by existing Federal laws and in compliance with the mandatory requirements for environmental protection in accordance with the local regulations.

8. EMS declaration

The instrument complies with the immunity and electromagnetic emission requirements of EN 61326-2-6: 2013.

The instrument is intended for use in the electromagnetic environment described below:

- The device was designed and tested in accordance with the requirements of CISPR 11 and it is suitable for use in all rooms, including ones, which are directly connected to the public low-voltage mains network.
- The quality of the electricity supply has to comply with typical conditions of use in commercial institutions or hospitals.
- Power frequency of magnetic fields has to be at a level corresponded with typical conditions of use in commercial institutions or hospitals.
- The instrument uses radiofrequency energy exclusively for its internal function. The level of radiofrequency emission is very low and does not lead to disturbances in the operation of electronic equipment located close to it.
- It is forbidden to use the instrument near sources of strong electromagnetic radiation that could interfere its normal functioning.

Notes:

1. The manufacturer is responsible for providing the consumer information about the electromagnetic compatibility of the instrument.
2. The customer is responsible for maintaining electromagnetic environment, which ensures instrument normal operation in accordance with its intended purpose.

9. Warranties

The manufacturer guarantees proper operation of the instrument and its conformity to TS 9443-004-96301278-2010 while meeting the requirements of this Operations Manual.

The warranty period for the instrument is 24 months from the date of its sale. Warranty repair is acceptable upon providing of warranty repair card along with the filled deficiency report.

Average life of the instrument is not less than 5 years from the start of its operation.

Guaranteed storage life while meeting storage conditions (indoor under temperature 5 ° C - 40 ° C) of the instrument is 12 months from the date of manufacture.

During the warranty period, the manufacturer bears responsibility to eliminate the instrument's defects free of charge by repairing or replacing it on a similar one, provided that the occurred defects are fault of the manufacturer.

Execution the warranty repair of the failed instrument by the manufacturer entails prolongation of the warranty period for the time of such repair.

The manufacturer is not responsible for compatibility of the provided software with any hardware or software supplied by other manufacturers, unless otherwise stipulated.

In no event shall the manufacturer and seller be liable for any losses, including loss of data, loss of profits and other incidental, consequential or indirect losses arising from improper installation, maintenance and operation or related to failure / temporary no operability of the instrument.

The manufacturer is not responsible for defects and malfunctions of the instrument that arising from the following:

- disregard of rules transportation, storage conditions, operation or improper installation;
- improper actions connected to improper usage of the instrument as well as disregard of the requirements set out in the operational manual;
- non authorized repair or changes in the design of instrument as well as cases of violation of warranty seals on the body of the instrument;
- force majeure (fire, flood, earthquake, etc.) or influence of some random external factors (power surges, etc.);
- Entering foreign objects (substances, liquids, insects, etc.) inside the instrument.

The warranty does not cover instruments that have external defects (obvious mechanical damage, cracks, cleavage on the body and inside, broken contacts of connectors and so on).

10. Packing Certificate

“DTprime” Real-Time PCR instrument,

Serial number _____ manufactured by

DNA-Technology, Research & Production, LLC has been packed in accordance with the TS 9443-004-96301278-2010 requirements.

Packaging date “.....”....., 20.....

The packaging is done by _____ (signature)

The packed product is accepted by _____ (signature)

L.S.

Note. The form is to be filled in at the factory that packed the product.

11. Acceptance certificate

“DTprime” Real-Time PCR instrument,

Serial number _____ manufactured by

DNA-Technology, Research & Production, LLC has passed the acceptance test, complies with TS 9443-004-96301278-2010 and has been qualified for operations.

Date of issue “.....”....., 20.....

L.S.

Signatures of officers responsible for the acceptance _____

12. Cards of warranty repair

CARD No. 1 *to be filled in by the manufacturer*

For warranty repair (maintenance) of DTprime Real-Time PCR instrument

..... manufactured by

(factory No. of the product) (date)

Representative of the manufacturer's quality control department

(QCD stamp)

Note of sale _____

(manufacturer's name)

".....", 20.....

manufacturer's stamp

(date) (personal signature)

Owner and his address

.....

(personal signature)

.....

CARD No. 2 *to be filled in by the manufacturer*

For warranty repair (maintenance) of DTprime Real-Time PCR instrument

..... manufactured by

(factory No. of the product) (date)

Representative of the manufacturer's quality control department

(QCD stamp)

Note of sale _____

(manufacturer's name)

".....", 20.....

manufacturer's stamp

(date) (personal signature)

Owner and his address

.....

(personal signature)

.....

Reverse of CARD No. 1 to be filled in by a service center

Product factory No.

Repair content
.....
.....
.....
.....

Date of repair.....

(day, month, year)

Serviceman..... Owner.....
(signature, stamp) (signature)

Reverse of CARD No. 2 to be filled in by a service center

Product factory No.

Repair content
.....
.....
.....
.....

Date of repair.....

(day, month, year)

Serviceman..... Owner.....
(signature, stamp) (signature)

Manufacturer: DNA-Technology, Research & Production, LLC
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E-mail: protvino@dna-technology.ru
<http://www.dna-technology.ru>

Seller: «DNA-Technology», LLC
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building 5, floor 1, office 12
Phone/fax: +7(495) 640-17-71
E-mail: info@dna-technology.com

Customer support:
Tel.: 8 800 200-75-15 (free for Russia)
E-mail: hotline@dna-technology.ru
Feedback form see on DNA-Technology's website
http://www.dna-technology.ru/customer_support/

Service department:
Tel.: +7(4967) 31-14-67, +7(4967) 31-06-71 (ex. 3126)
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