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**For professional use only**

HHV-6 REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE



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TABLE OF CONTENTS

1. INTENDED USE	3
2. METHOD.....	3
3. CONTENT	4
4. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED	5
5. TRANSPORT AND STORAGE CONDITIONS.....	6
6. WARNINGS AND PRECAUTIONS.....	7
7. SAMPLES	9
8. SAMPLES	10
9. CONTROLS	18
10. DATA ANALYSIS	19
11. SPECIFICATIONS	20
12. TROUBLESHOOTING	21
13. QUALITY CONTROL.....	22
14. KEY SYMBOLS	22

1. INTENDED USE

The **HHV-6 REAL-TIME PCR Detection Kit** is intended for research and diagnostic applications.

The **HHV-6 REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HHV-6 REAL-TIME PCR Detection Kit** is designed to detect Human herpesvirus 6 (HHV-6) nucleic acids in human biological samples (blood, cerebrospinal fluid, biopsy samples or punctate from lesions of organs and tissues, swabs from the oropharynx, saliva) by real-time PCR.

Indications: symptoms of infection caused by Human herpesvirus 6 (HHV-6).

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HHV-6 REAL-TIME PCR Detection Kit**.

The **HHV-6 REAL-TIME PCR Detection Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

Apply the kit only as directed in this instruction for use.

2. METHOD

The implemented PCR method is based on amplification of a target DNA sequence. The process of amplification includes repeating cycles of thermal DNA denaturation, annealing of primers with complementary sequences and their extension by DNA-polymerase.

To increase the sensitivity and specificity of the amplification reaction, the use of a hot-start is provided. Hot-start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin or the use of Taq-polymerase blocked by antibodies. The polymerase chain reaction starts only when paraffin is melted or thermal dissociation of a complex of Taq polymerase and antibodies is happened. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The PCR-mix contains two target-specific probes bearing reporter fluorescent dyes (Fam and Hex) and quencher molecules. Once hybridized to a target sequence, the probes become activated. As a result of activation fluorescence increases proportionally to target sequence amplification. The intensity of fluorescence is measured at every cycle of reaction with a Real-time PCR thermal cycler data collection unit and analyzed with the software provided.

The PCR-mix includes the Internal control (IC), which is intended to assess the quality of the polymerase chain reaction. DNA probe used for the detection of the HHV-6 product amplification includes fluorescent dye Fam. DNA probe used for the detection of the internal control amplification product includes the fluorescent dye Hex. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam/Green	Hex/Yellow	Rox/Orange	Cy5/Red	Cy5.5/Crimson
HHV-6	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite, DTprime or DTprime II real-time thermal cyclers for **HHV-6 REAL-TIME PCR Detection Kit** (see the catalogue at <https://www.dna-technology.com> to see available supply options). The current version of the software is available for download at <https://www.dna-technology.com/software>.

The **HHV-6 REAL-TIME PCR Detection Kit** is also approved for use with CFX96 (Bio-Rad), Rotor-Gene Q (Qiagen), and Applied Biosystems QuantStudio 5 (Life Technologies Pte. Ltd.) real-time thermal cyclers.

3. CONTENT

The **HHV-6 REAL-TIME PCR Detection Kit** comes in package S (tubes/strips) and package U. The detailed description of content is represented in Tables 2–4.

Table 2. The **HHV-6 REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P208-S3/9EU

Reagent	Description	Amount	Volume per tube
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	tubes, 12 strips of 8	20 µL in each
Taq polymerase solution	Colorless transparent liquid	2 tubes	500 µL in each
Mineral oil	Colorless transparent viscous oily liquid	2 tubes	1.0 mL in each
Positive control ¹	Colorless transparent liquid	1 tube	130 µL
Strip caps	12 strips of 8		

Table 3. The **HHV-6 REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P208-23/9EU

Reagent	Description	Amount	Volume per tube
Paraffin-sealed PCR mix	Colorless or pink transparent liquid under waxy white fraction	96 individual tubes	20 µL in each
Taq polymerase solution	Colorless transparent liquid	2 tubes	500 µL in each
Mineral oil	Colorless transparent viscous oily liquid	2 tubes	1.0 mL in each
Positive control ¹	Colorless transparent liquid	1 tube	130 µL

Table 4. The **HHV-6 REAL-TIME PCR Detection Kit** content, package U for R1-P208-UA/9EU

Reagent	Description	Amount	Volume per tube
PCR mix	Colorless or pink transparent liquid	1 tube	600 µL
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	1 tube	30 µL
PCR buffer	Colorless transparent liquid	1 tube	600 µL
Positive control ¹	Colorless transparent liquid	1 tube	130 µL

All components are ready to use and do not require additional preparation for operation.

The **HHV-6 REAL-TIME PCR Detection Kit** in package S is designed for 96 tests (no more than 24 runs), including analysis of test samples, negative controls and positive controls.

The **HHV-6 REAL-TIME PCR Detection Kit** in package U is designed for 96 tests with at least 5 samples per run (3 test samples, negative control and positive control).

¹ - marking as C+ is allowed

4. ADDITIONAL REAGENTS AND EQUIPMENT REQUIRED

The following equipment, reagents and consumables are required:

Reagents, equipment and consumables	Package S		Package U, dosing	
	strips ¹	tubes	manual	automated
UV PCR cabinet	•	•	•	•
Real-time detecting thermal cycler ²	•	•	•	•
Vortex mixer	•	•	•	•
Vortex rotor for 0.2 mL strips	•	-	-	-
Refrigerator with freezer	•	•	•	•
Tube rack for 0.2 mL tubes	-	•	•	-
Tube rack for 0.2 mL strip tubes	•	-	-	-
Tube rack for 1.5 mL tubes	•	•	•	•
Single channel pipettes (dispensers covering 2.0–20 µL; 20–200 µL; 200–1,000 µL volume range)	•	•	•	•
RNase and DNase free filtered pipette tips (volume 20 µL)	•	•	•	•
RNase and DNase free pipette tips (volume 200 µL; 1,000 µL)	•	•	•	•
Pipette rack	•	•	•	•
RNase and DNase free 1.5 mL microfuge tubes with caps	•	•	•	•
RNase and DNase free 0.2 mL PCR tubes with caps	-	-	•	-
Powder-free surgical gloves	•	•	•	•
Container for used pipette tips, tubes and other consumables	•	•	•	•
DTstream12 M1 or DTstream15 M1 liquid handling station	-	-	-	•
RNase and DNase free filter pipette tips (volume 200 µL) for DTstream* M1, or similar	-	-	-	•
DTpack plate sealing device	-	-	-	•
Centrifuge for microplates (RCF(g) at least 100)	-	-	-	•
Polymer thermal film for microplate sealing	-	-	-	•
96-well PCR microplate	-	-	•	•
384-well PCR microplate	-	-	-	•
Physiological saline solution 0.9% NaCl (sterile) (if necessary)				
Transport medium (if necessary), the following are recommended:				
– STOR-F transport medium for biomaterial samples				
NA extraction reagent kits ³ , the following are recommended:				
– PREP-NA;				
– PREP-GS;				
– PREP-RAPID;				
– PREP-OPTIMA;				
– PREP-MB MAX;				
– PREP-MB-RAPID II.				
Notes and specifications: ¹ - not used with Rotor-Gene Q detecting thermal cycler ² - hereinafter – detecting thermal cycler; the required parameters are indicated below ³ - possibility of DNA extraction reagent kit/set use for extraction of target microorganisms' DNA is determined by the biomaterial type (see 8.1) “•” – the piece of equipment/reagent is required “-” – the piece of equipment/reagent is not required				

The following detecting thermal cyclers are validated for work with the **HHV-6 REAL-TIME PCR Detection Kit**:

- DTprime in DTprime *M* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime;
- DTprime II in DTprime II *M* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime II;
- DTprime in DTprime *X* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime *X*(only for package U, automatic dosing using 384-well PCR microplates);
- DTprime II in DTprime II *X* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTprime II *X* (only for package U, automatic dosing using 384-well PCR microplates);
- DTLite in DTLite *S* modification (manufactured by “DNA-Technology R&P”, LLC), hereinafter – DTLite (only for package S and package U, manual dosing, tubes);
- Rotor-Gene Q (manufactured by QIAGEN GmbH, Germany), hereinafter – Rotor-Gene Q (only for package S, tubes, and package U, manual dosing, tubes);
- CFX96 (Optical Reaction Module CFX96) (manufactured by Bio-Rad Laboratories, USA), hereinafter – CFX96;
- Applied Biosystems QuantStudio 5 (manufactured by Life Technologies Pte. Ltd., Singapore), hereinafter – Applied Biosystems QuantStudio 5.

For the use of detecting thermal cyclers other than those listed in the table, please consult the reagent kit manufacturer.

Software:

The most recent version of the DT thermal cyclers software can be downloaded from <https://www.dna-technology.com/software>.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

5.1. Storage conditions

5.1.1. Package S

- All components of the **HHV-6 REAL-TIME PCR Detection Kit** must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mix must be stored out of light over the storage period.

5.1.2. Package U

- All components of the **HHV-6 REAL-TIME PCR Detection Kit**, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- PCR mix must be stored out of light over the storage period.
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 22°C to minus 18°C over the storage period.

WARNING! The excessive temperature and light can be detrimental to product performance.

5.2. Transport conditions

Transportation of the reagent kit is carried out in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container corresponding to the storage conditions of the kit components.

5.2.1. Package S

- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.

5.2.2. Package U

- It is allowed to transport the kit, except for TechnoTaq MAX polymerase, in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes from 2°C to 25°C for no longer than 5 days.
- It is allowed to transport TechnoTaq MAX polymerase in thermoboxes with ice packs by all types of roofed transport at the temperature inside the thermoboxes up to 25°C for no longer than 5 days.

WARNING! Reagent kits transported with violation of temperature conditions must not be used.

5.3. Shelf-life of the kit following the first opening of the primary container

5.3.1. Package S

- All components of the kit must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- Paraffin-sealed PCR mix must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.

5.3.2. Package U

- All components of the kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C over the storage period.
- PCR mix must be stored in a refrigerator or a cooling chamber at temperatures from 2°C to 8°C and out of light over the storage period.
- TechnoTaq MAX polymerase must be stored in a freezer at temperatures from minus 22°C to minus 18°C over the storage period.

WARNING! The kits stored under undue regime must not be used.

An expired **HHV-6 REAL-TIME PCR Detection Kit** must not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

Conformity of **HHV-6 REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Molecular biology procedures, such as nucleic acid extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous or invalid results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.
- Wear powder-free single-use surgical gloves. Wear work clothes and personal protective equipment while working with pathogenic microorganisms. The work clothes and personal protective equipment must be suitable for work to be performed and comply with health and safety requirements.

- Avoid any direct contact with the biological samples, reagents and materials used to carry out the test. Avoid producing spills or generating aerosols. Do not eat/drink components of the kit. Do not inhale gas/fumes/vapor/aerosols produced by the components of the kit. Avoid contact with eyes.
- Samples must be handled under a laminar flow hood.
- Pipettes used to handle samples must only be used for one purpose. The pipettes must be of positive displacement type or be used with aerosol barrier pipette tips.
- The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way to be utilized in a single session.
- Handle and dispose of all biological samples, reagents and materials used to carry out the assay as if infectious^{2, 3}. Any material being exposed to biological samples must be treated with disinfecting solution for at least 30 min or autoclaved for 1 hour at 121°C before disposal.
- All of the liquid solutions are designed for single use and cannot be used more than once in amplification reactions.
- Only use the reagents provided in the kit and those recommended by the manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits.
- All laboratory equipment and tools, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, gloves, etc., as well as reagents must be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Never introduce amplification products in the area designed for extraction/preparation of amplification reactions.
- Do not open the tubes after amplification. Avoid producing accidental spills of the amplification products. Dispose of all PCR waste materials (tubes, tips etc.) only in a closed form in a specialized sealed container with disinfectant solution. Waste materials must be removed in accordance with laboratory internal procedures, and with national and international standards.
- Working surfaces, as well as rooms where NA extraction and PCR are performed, must be disinfected with bactericidal irradiators (UVGI) for 30 min before and after the assay. All surfaces in the laboratory (test tube racks, equipment, tools, etc.) must be treated with disinfecting solution daily.

Emergency actions

Eye Contact: If any component of the kit enters the eyes, flush the eyes gently using potable running water for 15 min or longer, making sure that the eyelids are held open. If pain or irritation occurs, seek medical attention.

Skin Contact: If any component of this kit comes into contact with the skin and causes discomfort, remove any contaminated clothing. Rinse the affected area with plenty of soap and water. If pain or irritation occurs, seek medical attention.

Ingestion: If any component of this kit is ingested, rinse the mouth with plenty of potable water. If irritation or discomfort occurs, seek medical attention.

Do not use the kit:

- if the transportation and storage conditions have been violated;

² - All oligonucleotide components are produced by artificial synthesis in compliance with internal quality control protocol. They do not contain blood or products of blood processing.

³ - Positive control is produced using artificial DNA synthesis technology, it does not contain parts of infectious agents.

- if the appearance of the reagents does not correspond to the product documentation;
- if the packaging of the kit components is breached;
- after the expiry date of the kit.

Adverse health effects are **NOT** anticipated from routine use of this kit in compliance with the current instruction for use.

7. SAMPLES

The **HHV-6 REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from blood, cerebrospinal fluid, biopsy samples or punctate from lesions of organs and tissues, swabs from the oropharynx, saliva.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material.

7.1. General requirements

PCR analysis refers to direct methods of laboratory research; therefore, the collection of biological material must be carried out from the site of infection localization. The decision to examine a localization shall be taken by a physician based on the collected anamnesis and the clinical picture of the disease.

The quality of sampling, sample storage, transport, and pretreatment are of great importance for obtaining correct results.

If biomaterial from several biotopes is required, repeat the procedure, collecting biomaterial into a new tube each time.

Incorrect sampling may lead to invalid results and, therefore, to the necessity for repeated sampling.

Use RNase and DNase free filtered tips during biomaterial preparation and NA extraction.

To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube.

7.2. Interfering substances

The presence of PCR inhibitors in a sample may cause invalid results. The sign of PCR inhibition is the simultaneous absence of internal control and specific product of amplification.

PCR inhibitors are hemoglobin and medications present in the DNA sample as a result of incomplete removal during the extraction of DNA from a biomaterial sample, as well as the presence of isopropyl alcohol and methyl acetate in the DNA sample as a result of incomplete removal of wash solutions during sample preparation.

The maximum concentration of interfering substances, which do not affect the amplification of the laboratory control sample and internal control: hemoglobin – 0.35 mg/mL of the DNA sample, isopropyl alcohol – 100 µL/mL DNA sample, methyl acetate – 100 µL/mL of the DNA sample.

To assess possible interference of medications, those potentially present in leftover quantities (Miramistin®, chlorhexidine bigluconate) in human biomaterial samples from the corresponding biotopes were selected.

For all tested medicinal products, no effect was demonstrated at concentrations of up to 10% in the biomaterial sample.

7.3. Sample collection

WARNING! Sample preparation may be required before DNA extraction!

7.3.1 Peripheral blood

Biomaterial collection is carried out in accordance with instructions for use to the NA extraction kits (see 8.1).

Method limitations⁴: intravenous heparin injections, infusions of parenteral nutrition less than 6 hours before the test.

7.3.2 Cerebrospinal fluid, saliva

Biomaterial collection is carried out in accordance with instructions for use to the NA extraction kits (see 8.1).

7.3.3 Biopsy samples/punctate

Biomaterial collection is carried out in accordance with instructions for use to the NA extraction reagent kits (see 8.1).

If using **PREP-NA** and **PREP-GS** extraction kits: Biomaterial is collected into 1.5 mL disposable tubes with transport medium.

7.3.4 Swabs from the oropharynx

Biomaterial collection is carried out in accordance with instructions for use to the NA extraction kits (see 8.1).

Method limitations⁵: local application of medicines (sprays, drops, creams, ointments) less than 24 hours before the test. If using aerosols and other inhalation medicines for bronchial asthma treatment, biomaterial should be collected no earlier than 3 hours after the inhalation.

WARNING! Take material into tubes with **PREP-RAPID** reagent using a dry swab! Solutions **must not** contact with skin, eyes and mucous membranes.

7.4. Transport and storage of samples

Transport and storage conditions of biomaterial samples are stated in the instructions for use of the NA extraction reagent kits or the transport media used for transport and storage of samples (see 8.1).

Samples may be transported and stored in physiological saline at temperatures from 2°C to 8°C no more than 24 hours prior to analysis. When it is impossible to deliver the material in the laboratory during the day, a one-time freezing of the material is allowed. The frozen material is allowed to be stored at temperatures from minus 22°C to minus 18°C for one month (if it does not contradict the requirements to the used NA extraction reagent kits/sets).

WARNING! Avoid repeated freezing and thawing of samples.

7.5. Biomaterial preparation for DNA extraction

Biomaterial preparation (if necessary) is performed in accordance with the instructions for use for the NA extraction reagent kits.

8. SAMPLES

8.1 DNA extraction from biological material

NA extraction is performed in accordance with the instructions for use of the corresponding reagent kit.

DNA extraction is carried out according to the extraction kit instructions. **PREP-NA**, **PREP-GS**, **PREP-RAPID**, **PREP-OPTIMA**, **PREP-MB MAX**, and **PREP-MB-RAPID II** extraction kits are recommended.

⁴ - If it does not contradict the requirements of the NA extraction kit.

⁵ - If it does not contradict the requirements of the NA extraction kit.

Table 5. Reagent kits recommended for DNA extraction for further HHV-6 test

Reagent kit	Biomaterial	Minimum eluate volume, μ L
PREP-NA	Cerebrospinal fluid, biopsy samples/punctate, swabs from the oropharynx, saliva	50
PREP-GS	Cerebrospinal fluid, biopsy samples/punctate, swabs from the oropharynx, saliva	100
PREP-RAPID	Cerebrospinal fluid, swabs from the oropharynx, saliva	500
PREP-OPTIMA	Whole peripheral blood ⁶	100
	Cerebrospinal fluid, biopsy samples/punctate, Swabs from the oropharynx	400
PREP-MB MAX	Whole peripheral blood	50
PREP-MB-RAPID II	Swabs from the oropharynx	100

General requirements:

- Follow the DNA preparation recommendations from the NA extraction kit's instruction.
- Use filter tips.
- Open tube caps only when introducing the DNA sample, then close immediately.
- If using strips: close the strip cap after introducing samples before proceeding to the next strip.
- Ensure all tubes are tightly closed after dosing.

For DNA preparations after storage, prepare them as follows before adding to the amplification mixture:

- **PREP-NA, PREP-RAPID, PREP-OPTIMA:** vortex (3–5 sec), then centrifuge on vortex (1–3 sec).
- **PREP-GS:** vortex (5–10 sec), incubate at 50 °C (5 min) (if the eluate was refrigerated), then centrifuge at 16,000 RCF(g) (1 min).
- **PREP-MB MAX, PREP-MB-RAPID II:** centrifuge on vortex carefully (without shaking, 1–3 sec), place into magnetic rack. If supernatant transferred to new tubes — centrifuge (1–3 sec) after shaking.

WARNING!

- Run a negative control alongside DNA extraction through all preparation stages. Use physiological saline solution (in volumes per the extraction kit instructions) or the kit provided negative control.
- DNA extraction from biopsy samples/punctate using **PREP-NA** and **PREP-GS** DNA extraction kits is carried out according to the instruction below.

8.2 DNA extraction from biopsy samples/punctate using PREP-NA and PREP-GS DNA extraction kits

8.2.1 General requirements

- 8.2.1.1. Use RNase and DNase free filtered tips during biomaterial preparation and NA extraction. Change the tip after each solution removal from the tube. If using aspirator, use RNase and DNase free non-filtered tips.
- 8.2.1.2. When adding the solution into the tube with biomaterial, add solution carefully, without

⁶ - only if using PREP-OPTIMA MAX reagent kit

touching the walls of the tube. If touching occurred, change the tip.

8.2.1.3. To prevent contamination, only open the cap of the tube you are working with and close it before proceeding to the next tube. Working with several tubes with open caps simultaneously is prohibited.

8.2.1.4. Test samples and negative control (C-) must be treated similarly according to this instruction.

8.2.2 DNA extraction from biopsy samples/punctate using PREP-NA DNA extraction kit

WARNING!

1. Before starting work it is necessary to:

- preheat the thermostat to 65°C;
- take out of the refrigerator the NA extraction reagent kit and check the absence of precipitate in the lysing solution. In case of precipitation it is necessary to heat the vial with lysis solution on the thermostat preheated to 65°C, until complete dissolution of the precipitate. Then stir the solution by turning the vial upside down 5-10 times, avoid foaming. Before use, cool the solution to room temperature (18°C to 25°C). The precipitate can also be dissolved at room temperature (18°C to 25°C) within approximately 12 hours.

2. Tube caps may open during heating! Use tubes with self-lock caps (e.g. Eppendorf Safe-Lock Tubes) or programmable thermostats with clamp cover.

8.2.2.1. Mark a 1.5 mL plastic tube for each test sample and negative control (C-).

8.2.2.2. Add 300 µL of lysis solution into each tube with biopsy/punctate samples and into the C- tube. Avoid touching the walls of the tubes.

8.2.2.3. Add 100 µL of negative control into the C- tube. Close the tubes tightly and shake on vortex for 3–5 sec.

8.2.2.4. Heat the tubes on thermostat at 65°C for 30 min, spin the tubes on vortex for 3–5 sec.

8.2.2.5. Transfer supernatant into the corresponding marked tubes for test samples. Do not transfer supernatant into the C- tube.

8.2.2.6. Add 400 µL of precipitation buffer into each tube, avoid touching the walls of the tubes. Shake on vortex for 3–5 sec.

8.2.2.7. Centrifuge the tubes at RCF(g) 12,000–16,000 for 15 min.

8.2.2.8. Remove supernatant as fully as possible using separate tip for each tube. Avoid touching the precipitate.

8.2.2.9. Add 500 µL of wash solution No. 1 to the precipitate, close the tubes and stir by turning tubes gently upside down 3–5 times.

8.2.2.10. Centrifuge the tubes at RCF(g) 12,000–16,000 for 5 min.

8.2.2.11. Remove supernatant fully using separate tip for each tube. Avoid touching the precipitate.

8.2.2.12. Add 300 µL of wash solution No. 2 to the precipitate, close the tubes and stir by turning tubes gently upside down 3–5 times.

8.2.2.13. Centrifuge the tubes at RCF(g) 12,000–16,000 for 5 min.

8.2.2.14. Remove supernatant fully using separate tip for each tube. Avoid touching the precipitate.

8.2.2.15. Open the tubes and dry the precipitate at 65°C for 5 min.

8.2.2.16. Add 50 µL of dilution buffer, close the tubes.

8.2.2.17. Spin down the drops on vortex for 1–3 sec.

8.2.2.18. Heat the tubes on thermostat at 65°C for 10 min. Shake the tubes on vortex

for 3–5 sec.

8.2.2.19. Centrifuge the tubes at RCF(g) 12,000–16,000 for 30 sec.

DNA preparation is ready to be introduced into the PCR mix.

DNA preparation can be stored at temperature from minus 22°C to minus 18°C for up to 1 month or at temperature from minus 72°C to minus 68°C for up to 1 year.

Before using DNA preparation for PCR after storage, thaw DNA preparation and negative control at room temperature (from 18°C to 25°C) or at temperature from 2°C to 8°C, then shake the tubes with DNA preparation and negative control on vortex for 3–5 sec and centrifuge on vortex for 1–3 sec.

DNA preparation is ready to be introduced into the PCR mix.

WARNING! Only one thawing is allowed for DNA preparation!

8.2.3 DNA extraction from biopsy samples/punctate using PREP-GS DNA extraction kit

WARNING! Before starting work, preheat the thermostat to 50°C.

- 8.2.3.1. Mark a 1.5 mL plastic tube for each test sample and negative control (C-).
- 8.2.3.2. Add 150 µL of lysis solution into each tube with biopsy/punctate samples and into the C- tube. Avoid touching the walls of the tubes.
- 8.2.3.3. Add 50 µL of sterile physiological solution or transport medium into the C- tube. Close the tubes tightly and shake on vortex for 3–5 sec.
- 8.2.3.4. Heat the tubes on thermostat at 50°C for 30 min, spin the tubes on vortex for 3–5 sec.
- 8.2.3.5. Transfer supernatant into the corresponding marked tubes for test samples. Do not transfer supernatant into the C- tube.
- 8.2.3.6. Resuspend sorbent thoroughly on vortex. Turn the tube upside down and make sure the sorbent has not stuck to the bottom of the tube. Mix the sorbent again, if necessary.
- 8.2.3.7. Add 20 µL of resuspended sorbent into each tube, including C-, avoid touching the walls of the tubes. Close the tubes and shake on vortex for 3–5 sec.
- 8.2.3.8. Centrifuge the tubes at RCF(g) 12,000–16,000 for 1 min.
- 8.2.3.9. Remove supernatant fully using separate tip for each tube. Avoid touching the precipitate.
- 8.2.3.10. Add 200 µL of wash solution No. 1 to the precipitate, close the tubes and shake on vortex for 3–5 sec.
- 8.2.3.11. Centrifuge the tubes at RCF(g) 12,000–16,000 for 1 min.
- 8.2.3.12. Remove supernatant fully using separate tip for each tube. Avoid touching the precipitate.
- 8.2.3.13. Add 200 µL of wash solution No. 2 to the precipitate, close the tubes and shake on vortex for 3–5 sec.
- 8.2.3.14. Centrifuge the tubes at RCF(g) 12,000–16,000 for 1 min.
- 8.2.3.15. Add 200 µL of wash solution No. 3 to the precipitate, close the tubes and shake on vortex for 3–5 sec.
- 8.2.3.16. Centrifuge the tubes at RCF(g) 12,000–16,000 for 1 min.
- 8.2.3.17. Remove supernatant fully using separate tip for each tube. Avoid touching the precipitate.
- 8.2.3.18. Open the tubes and dry the precipitate at 50°C for 5 min.
- 8.2.3.19. Add 100 µL of elution solution to the precipitate. Avoid touching the walls of the tube. Close the tubes and shake on vortex for 5–10 sec.
- 8.2.3.20. Heat the tubes and at 50°C for 5 min.

8.2.3.21. Centrifuge the tubes at RCF(g) 12,000–16,000 for 1 min.

Supernatant containing the extracted DNA is ready to be introduced into the PCR mix.

DNA preparation can be stored up to 7 days at temperature from 2°C to 8°C. Before using DNA preparation for PCR, repeat 8.2.3.20— 8.2.3.21.

If sample is to be stored for more than 7 days, transfer supernatant into a separate disposable tube. Store at temperatures from minus 22°C to minus 18°C for up to 6 months.

Before using DNA preparation for PCR after storage, thaw DNA preparation and negative control at room temperature (from 18°C to 25°C) or at temperature from 2°C to 8°C, then shake the tubes with DNA preparation and negative control on vortex for 3–5 sec and centrifuge on vortex for 1–3 sec.

DNA preparation is ready to be introduced into the PCR mix.

WARNING! Only one thawing is allowed for DNA preparation!

8.3 Preparing PCR for package S

WARNING!

- The reagents and tubes should be kept away from direct sunlight.
- When using package S, strips, strictly observe the completeness of the strips and caps. Do not use the caps for the strips of the other kits!

8.3.1. Mark one tube/strip tube with the paraffin-sealed PCR mix for each test sample, C-, and C+.

WARNING! The volume of reagents is calculated for no more than 24 runs assuming a variable number of test samples, 1 negative control and 1 positive control per run.

Example: To test 4 samples, mark 4 tubes for samples, one C- tube and one C+ tube. Total number of tubes is 6.

8.3.2. Shake the tubes with Taq polymerase solution on vortex, then spin down the drops.

8.3.3. Add **10 µL** of Taq polymerase solution to each tube. Avoid paraffin layer break.

WARNING! If using Rotor-Gene Q detecting thermal cycler, do not add mineral oil into the tubes.

8.3.4. Add one **drop** of mineral oil (~20 µL) to each tube. Cover the tubes/strips loosely with caps.

8.3.5. Shake the tube with C+ on vortex, then spin down the drops.

8.3.6. Add **5.0 µL** of DNA sample into corresponding tubes. Do not add DNA into the C- and C+ tubes. Avoid paraffin layer break.

8.3.7. Add **5.0 µL** of C- which passed whole DNA extraction procedure into the C- tube. Avoid paraffin layer break.

8.3.8. Add **5.0 µL** of C+ into the corresponding tube. Avoid paraffin layer break.

8.3.9. Thoroughly spin down the drops on vortex (if using Rotor-Gene Q detecting thermal cycler, spinning is not required).

8.3.10. Set the tubes/strips into the real-time thermal cycler.

8.3.11. **For DT detecting thermal cyclers:**

Launch the operating software for DT instrument⁷. Add corresponding test⁸, specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes/strips in thermal unit (see 8.3.10) and run PCR. See Table 6.

⁷ - Please, apply to Operation Manual for DTprime, DTprime II and DTLite Real-Time PCR instruments PART II.

⁸ - The test for DT detecting thermal cyclers is created by entering parameters or is provided by the kit manufacturer.

8.3.12. For Rotor-Gene Q, CFX96, Applied Biosystems QuantStudio 5 detecting thermal cyclers:

Perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 7, 8, 9, respectively.

Table 6. The PCR program for DTLite, DTprime and DTprime II thermal cyclers (package S)

Step	Temperature, °C	Min.	Sec.	Number of cycles	Optical measurement	Type of the step
1	80	0	30	1		Cycle
	94	1	30			
2	94	0	30	5		Cycle
	64	0	15		√	
3	94	0	10	45		Cycle
	64	0	15		√	
4	94	0	5	1		Cycle
5	25 ⁹	...	...	Holding		Holding
√ – optical measurement						

Table 7. The PCR program for Rotor-Gene Q thermal cyclers (package S, tubes)

Cycling	Temperature, °C	Hold Time, s	Cycle Repeats
Cycling	80 deg	60	1 time
	94 deg	90	
Cycling 2	94 deg	30	5 times
	57 deg v	15	
Cycling 3	94 deg	10	45 times
	57 deg v	15	
v—optical measurements, set the fluorescence measurement (Acquiring) on the Green (Fam), and Yellow (Hex) channels at 57°C			

Table 8. The PCR program for CFX96 thermal cyclers (packages S, U)

Step	Temperature, °C	Time, min:sec	Number of cycles
1	80	01:00	1
2	94	01:30	1
3	94	0:15	50
4	64 √	0:20	
√ – optical measurements (Plate Read), set the fluorescence measurement on the Fam, Hex channels at 64°C			

⁹ - Holding at 10°C is allowed.

Table 9. The PCR program for Applied Biosystems QuantStudio 5 thermal cyclers (packages S, U)

Stage	Step	Temperature, °C	Time, min:sec	Number of cycles
Holding	1	80	01:00	1
	2	94	01:30	1
PCR	1	94	0:20	50
	2	64 √	0:20	
√ – data collection for the necessary fluorophores (Fam, Vic (Hex)) is on				

8.4 Preparing PCR for package U, manual dosing

WARNING!

- The reagents and tubes must be kept away from direct sunlight.
- For amplification use 0.2 mL single-use amplification tubes or 96-well PCR plates¹⁰, sealed hermetically with thermal film. It is not recommended to use strips due to postamplification contamination hazard.

8.4.1 **Mark** the required number of 0.2 mL tubes or a 96-well plate for each test sample, C- and C+.

Note. It is recommended to test at least 5 samples per test (3 test samples, C- and C+).

Example: to test 4 samples, mark 4 tubes/reserve 4 wells for samples, 1 tube/well for C- and 1 tube/well for C+. The resulting number of tubes/wells is 6.

8.4.2 Shake the tubes with PCR mix on vortex, then spin down the drops.

8.4.3 Add **6.0 μ L** of PCR mix to each marked tube/well (including C- and C+).

8.4.4 Shake tubes with PCR buffer and TechnoTaq MAX polymerase on vortex, spin down the drops.

WARNING! Take TechnoTaq MAX polymerase out from the freezer immediately prior to use.

8.4.5 Prepare the mix of PCR buffer and TechnoTaq MAX polymerase. Add into the one tube:

6.0 x (N+1) μ L of PCR buffer,

0.3 x (N+1) μ L of TechnoTaq MAX polymerase,

where N is the quantity of samples to be tested taking into account C-, C+.

Example: to test 4 samples, C- and C+ in one PCR run, mark 6 tubes/reserve 6 wells (4 tubes/wells for test samples, 1 tube/well for C- and 1 tube/well for C+). Prepare the mix of PCR buffer and Taq polymerase for 7 (6+1) tubes/wells. Mix 42 μ L of PCR buffer and 2.1 μ L of TechnoTaq MAX polymerase.

8.4.6 Vortex the tubes with the mix of PCR buffer and TechnoTaq MAX and spin down the drops.

WARNING! Mix of PCR buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.4.7 Add **6.0 μ L** of the mix of PCR buffer and TechnoTaq MAX polymerase into each tube/well with PCR mix. Cover the tubes loosely.

WARNING! Follow the steps listed in pp. 8.2.8–8.2.14 **within two hours** after adding the mix of PCR buffer and TechnoTaq MAX polymerase to PCR mix.

8.4.8 Shake the tubes with C+ on vortex, then spin down the drops.

8.4.9 Add **6.0 μ L** of DNA sample into corresponding tubes/wells. Do not add DNA into the C-, C+ tubes/wells.

8.4.10 Add **6.0 μ L** of C- which passed whole DNA extraction procedure into the corresponding tube/well.

8.4.11 Add **6.0 μ L** of C+ into the corresponding tube/well.

8.4.12 In case of using **96-well PCR microplates**:

8.4.12.1. Place the plate carefully, without shaking into the DTpack sealing device.

¹⁰ - 96-well plates are not used with DTlite and Rotor-Gene Q detecting thermal cyclers

8.4.12.2. Seal the PCR plate with polymer thermal film according to the DTpack operation manual.

8.4.12.3. Centrifuge the plate at **RCF(g) 100 for 30 sec.**

8.4.13 In case of using **tubes**:

Centrifuge the tubes for 3–5 sec on vortex mixer (if using Rotor-Gene Q detecting thermal cycler, centrifugation is not required).

8.4.14 Set the tubes/microplate into the real-time thermal cycler.

8.4.15 **For DT detecting thermal cyclers:**

Launch the operating software for DT instrument¹¹. Add corresponding test¹², specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes/microplate in thermal unit (see 8.4.13) and run PCR. See Table 10.

8.4.16 **For CFX96, Applied Biosystems QuantStudio 5, Rotor-Gene Q detecting thermal cyclers:**

Perform PCR considering reaction mixture volume of 18 µL according to amplification programs shown in Tables 8, 9, 11, respectively.

Table 10. The PCR program for DTlite, DTprime and DTprime II thermal cyclers (package U)

Step	Temperature, °C	Min	Sec	Number of cycles	Optical measurement	Type of the step
1	80	0	5	15		Cycle
	94	0	5			
2	94	5	00	1		Cycle
3	94	0	30	5		Cycle
	64	0	15		√	
4	94	0	10	45		Cycle
	64	0	15		√	
5	94	0	5	1		Cycle
6	25 ¹³			Holding		Holding
√ – optical measurements						

Table 11. The PCR program for Rotor-Gene Q thermal cycler (package U)

Cycling	Temperature, °C	Hold Time, s	Cycle Repeats
Cycling	80 deg	60	1 time
	94 deg	300	
Cycling 2	94 deg	30	5 times
	57 deg v	15	
Cycling 3	94 deg	10	45 times
	57 deg v	15	

v – optical measurements, set the fluorescence measurement (Acquiring) on the Green (Fam), and Yellow (Hex) channels at 57°C

¹¹ - Please, apply to Operation Manual for DTprime, DTprime II and DTlite real-time PCR instruments PART II.

¹² - The test for DT detecting thermal cyclers is created by entering parameters or is provided by the kit manufacturer.

¹³ - Holding at 10 °C is allowed.

8.5 Preparing PCR for package U, using DTstream

WARNING!

- The reagents and tubes should be kept away from direct sunlight.
- For amplification use 96-well or 384-well¹⁴ PCR microplates hermetically sealed with thermal film.

Note – It is recommended to test at least 5 samples in 1 run (3 test samples, negative control and positive control).

8.5.1 Shake the tube with PCR mix on vortex, then spin down the drops.

8.5.2 Shake the tube with PCR buffer and TechnoTaq MAX polymerase on vortex, then spin down the drops.

WARNING! TechnoTaq MAX polymerase should be got out from the freezer immediately prior to use.

8.5.3 Prepare the mix of PCR buffer and TechnoTaq MAX polymerase according to the software for DTstream.

8.5.4 Shake the tube with the mix of PCR buffer and TechnoTaq MAX polymerase on vortex, then spin down the drops.

8.5.5 Shake the tubes with C+ on vortex, then spin down the drops.

8.5.6 Set the tubes with PCR mix, the mix of PCR buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls, and PCR microplate on the DTstream working table and **conduct dosage** of the components according to DTstream user manual.

8.5.7 After the end of dosing program on DTstream put the PCR microplate without shaking on the working table of DTpack sealing device.

8.5.8 Seal the PCR microplate according to the user manual of DTpack sealing device.

8.5.9 Centrifuge the microplate at **RCF(g) 100 for 30 sec.**

8.5.10 Set the PCR microplate into the real-time thermal cycler.

8.5.11 **For DT detecting thermal cyclers:**

Launch the operating software for DT instrument¹⁵. Add corresponding test¹⁶, specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes/plates in thermal unit (see 8.5.10) and run PCR. See Table 11.

8.5.12 **For CFX96 and Applied Biosystems QuantStudio 5 detecting thermal cyclers:**

Perform PCR considering reaction mixture volume of 18 µL according to amplification programs shown in Tables 8, 9, respectively.

9. CONTROLS

The **HHV-6 REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the HHV-6 genome. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing.

The PCR-mix from the kit includes the Internal control (IC). IC is an artificial plasmid intended to assess the quality of PCR performance.

To reveal possible contamination a negative control is required.

WARNING! A negative control sample should go through all stages of DNA extraction. Physiological saline

¹⁴ - Only for DTprime *X* and DTprime II *X* detecting thermal cyclers.

¹⁵ - Please, apply to Operation Manual for DTprime, DTprime II and DTlite real-time PCR instruments PART II.

¹⁶ - The test for DT detecting thermal cyclers is created by entering parameters or is provided by the kit manufacturer.

solution or negative control sample from an extraction kit can be used as a negative control in volumes as instructions.

For **HHV-6 REAL-TIME PCR Detection Kit** the test result is considered valid when:

- the exponential growth of the fluorescence level for the specific product is present, in this case the internal control is not taken into account;
- the exponential growth of the fluorescence level for the specific product is absent and for internal control is present.

For **HHV-6 REAL-TIME PCR Detection Kit** the test result is considered invalid when the exponential growth of the fluorescence level for the specific product and for internal control is not observed.

If positive control (C+) does **not** express growing fluorescence of the specific product or positive result, it is required to repeat the whole test. It may be caused by inhibitors, operation error or violation of storage and handling.

If negative control (C-) expresses growing fluorescence of the specific product or positive result, all tests of the current batch are considered false. Decontamination is required.

10. DATA ANALYSIS

10.1. Registration of the results is carried out automatically during amplification by the software provided with detecting thermocycler.

10.2. When using CFX96 detection thermal cyclers, use regression type analysis (Cq Determination Mode: Regression). In the “Baseline Setting” tab select “Baseline Subtraction Curve Fit”.

10.3. Result interpretation is carried out according to Table 12. The results are valid if the conditions for the interpretation of results obtained for control samples are met.

Table 12. PCR results interpretation

Fam/Green (target DNA), Cp/Cq/Ct	Hex/Yellow/Vic (IC), Cp/Cq/Ct	Result interpretation
Test samples		
Specified	Not considered	HHV-6 DNA is detected
Not specified	Specified	HHV-6 DNA is not detected
Not specified	Not specified	Invalid result
Negative control		
Not specified	Specified	Negative result Run results are valid
Positive control		
Specified	Not considered	Positive result Run results are valid

10.4. Invalid result may be due to the presence of inhibitors in the NA preparation obtained from biological material; incorrect execution of the analysis protocol, noncompliance with the amplification temperature regime, etc. In this case it is necessary to repeat PCR with the available DNA preparation, or to re-extract DNA and perform PCR for this sample, or to re-collect biological material from the patient (performed sequentially).

10.5. If a positive result is obtained for C-, the results of the whole batch should be considered invalid. In this case special measures are required for detection and elimination of possible contamination.

10.6. If a negative result is obtained for C+, the results of the whole batch should be considered invalid. In this case it is required to repeat PCR for the whole batch of samples.

11. SPECIFICATIONS

a. Analytical specificity

The samples with Human herpesvirus 6 DNA are to be registered positive for specific product (a fragment of the Human herpesvirus 6 genome) on Fam/Green channel. The samples free of Human herpesvirus 6 DNA are to be registered negative for specific product and positive for internal control on Hex/Yellow detection channel.

There are not non-specific positive results of amplification of DNA sample in the presence of Cytomegalovirus, Herpes simplex virus 1,2, Epstein-Barr virus, Human herpesvirus 8, Varicella-zoster virus, Human Papillomavirus 6, Human Papillomavirus 11, as well as human DNA in concentrations up to 1.0×10^8 copies/mL of the sample.

b. Analytical sensitivity (limit of detection)

LoD is 5 copies of HHV-6 DNA per amplification tube. LoD is determined by the analysis of serial dilutions of the laboratory control (LC).

Sensitivity of 5 copies per amplification tube corresponds to the following values of the DNA concentration of HHV-6 in case of using the following DNA extraction kits produced:

Biomaterial	DNA extraction reagent kit	Obtained preparation volume, μL	LoD, copies/sample
Swab from the oropharynx in 500 μL of transport medium ¹⁷	PREP-NA	50	50
	PREP-GS	100	100
	PREP-RAPID	500	500
	PREP-OPTIMA	400	400
	PREP-MB-RAPID II	100	100
Cerebrospinal fluid (extraction from 500 μL of sample)	PREP-NA	50	50
	PREP-GS	100	100
	PREP-RAPID	500	500
Cerebrospinal fluid (extraction from 1.0 mL of sample)	PREP-OPTIMA	400	400
Saliva (extraction from 500 μL of sample)	PREP-NA	50	50
	PREP-GS	100	100
	PREP-RAPID	500	500
Biopsy samples/punctate	PREP-NA	50	50
	PREP-GS	100	100
	PREP-OPTIMA	400	400
Whole peripheral blood (500 μL ¹⁸)	PREP-OPTIMA MAX	100	100
Whole peripheral blood (100 μL)	PREP-MB MAX	50	50

¹⁷ - STOR-F (DNA-Technology, Russia)

¹⁸ - If adding 100 μL of lysis solution

c. Diagnostic characteristics

Biomaterial	Diagnostic sensitivity	Diagnostic specificity
Blood	100 % (95 % CI: 86.28 %–100 %)	100 % (95 % CI: 86.28 %–100 %)
Cerebrospinal fluid	100 % (95 % CI: 86.28 %–100 %)	100 % (95 % CI: 86.28 %–100 %)
Biopsy samples or punctate from lesions of organs and tissues	100 % (95 % CI: 86.28 %–100 %)	100 % (95 % CI: 86.28 %–100 %)
Swab from the oropharynx	100 % (95 % CI: 86.28 %–100 %)	100 % (95 % CI: 86.28 %–100 %)
Saliva	100 % (95 % CI: 86.28 %–100 %)	100 % (95 % CI: 86.28 %–100 %)
Total	100 % (95 % CI: 97.09 %–100 %)	100 % (95 % CI: 97.09 %–100 %)

d. Reproducibility and repeatability

Reproducibility is 100%.

Repeatability is 100%.

WARNING! The claimed specifications are guaranteed when DNA extraction is performed with **PREP-RAPID**, **PREP-NA**, **PREP-GS**, **PREP-OPTIMA**, **PREP-MB MAX**, and **PREP-MB RAPID II** extraction kits.

12. TROUBLESHOOTING

Table 9. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handling requirements	Repeat whole test Dispose current batch
C-	+	Contamination	Dispose current batch Perform decontamination procedures
IC	Invalid	PCR inhibition	Repeat whole test Resample

If you face to any undescribed issues contact our customer service department regarding quality issues with the kit:

Phone: +7(495) 640.16.93

E-mail: hotline@dna-technology.ru

<https://www.dna-technology.com/support>

13. QUALITY CONTROL

The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service by quality issues of **HHV-6 REAL-TIME PCR Detection Kit** Technical support.

Technical support:

E-mail: hotline@dna-technology.ru

<https://www.dna-technology.com>

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

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<https://www.dna-technology.com>

14. KEY SYMBOLS

	<i>In vitro</i> diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
	Contains sufficient for <n> tests		Catalogue number
	Use-by date		Manufacturer
	Batch code		Keep away from sunlight
	Non-sterile		



R1-P208-S3/9EU
R1-P208-23/9EU
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