DNA-TECHNOLOGY

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IVD

For professional use only

HHV8 REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

EC REP

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R1-P203-23/9EU
R1-P203-UA/9EU

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1. INTENDED USE

The **HHV8 REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HHV8 REAL-TIME PCR Detection Kit** is designed to detect HHV8 nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: blood, bioptate or punctate from foci of organ and tissue lesions.

Indications: symptoms of infection caused by HHV8, monitoring the effectiveness of antiviral treatment, differential diagnosis of infections with similar clinical manifestations, in the complex examination of patients with lymphoproliferative diseases and hematoblastosis, examination of organ and tissue recipients before and after transplantation.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HHV8 REAL-TIME PCR Detection Kit.**

The **HHV8 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.

It is necessary to 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 HHV8 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
HHV8	IC	-	-	-

The automatic analysis is available on “DNA-Technology” made instruments: DTlite or DTprime REAL-TIME Thermal Cyclers for **HHV8 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 **HHV8 REAL-TIME PCR Detection Kit** is also approved for use with Rotor-Gene Q (Qiagen) real-time thermal cyclers.

3. CONTENT

The **HHV8 REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. The **HHV8 REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P203-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	12 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control*	Colorless transparent liquid	130 µL	1 tube
Strip's caps	12 8-caps		

* - marking as C+ is allowed

Table 3. The **HHV8 REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P203-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under waxy white fraction	1920 µL (20 µL in each tube)	96 tubes
Taq-polymerase solution	Colorless transparent liquid	1000 µL (500 µL in each tube)	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	2.0 mL (1.0 mL in each tube)	2 tubes
Positive control	Colorless transparent liquid	130 µL	1 tube

* - marking as C+ is allowed

Table 4. The **HHV8 REAL-TIME PCR Detection Kit** content, package U for R1-P203-UA/9EU

Reagent	Description	Total volume	Amount
PCR-mix	Colorless or slightly pink transparent liquid	600 µL	1tube
TechnoTaq MAX polymerase	Colorless transparent viscous liquid	30 µL	1 tube
PCR-buffer	Colorless transparent liquid	600 µL	1 tube
Positive control	Colorless transparent liquid	130 µL	1 tube

* - marking as C+ is allowed

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

The kit is intended for single use and designed for 96 tests (package S) including no more than 94 experimental samples, negative control and positive control samples. The kit in the package U is intended for 96 samples and requires no less than 5 samples in a single run (3 experimental samples, positive and negative controls) or on using a dosing device it is possible to run 96 tests simultaneously (94 experimental samples, negative control and positive control samples).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Single-use sterile flasks and sterile containers to collect clinical material;
- Sterile tubes containing transport media: "DNA-Technology" **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU) or equivalent for the transportation of the sample;
- For blood collection: 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- High speed centrifuge (RCF(g) no less than 16000) for 1.5 mL tubes;
- High speed centrifuge (RCF(g) no less than 1150) for 4.5 mL tubes;
- Solid-state thermostat (temperature range 25-98 °C);
- Vortex mixer;
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- RNase and DNase free pipette tips for aspirator with trap flask;
- Single channel pipettes (dispensers covering 20-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 1000 µL);
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-NA** (REF P-002/1EU), **PREP-GS** REF P-003/1EU), **PREP-RAPID** (REF P-001/1EU) (not applicable to male urethral swabs) and **PREP-MB RAPID (** REF P-116-N/4EU, P-116-A/8EU) extraction kits are recommended);
- Physiological saline solution 0.9% NaCl (Sterile);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Preamplification-reagent preparation area:

- UV PCR cabinet;

- Refrigerator;
- Freezing chamber (using detection kit in the package U REF R1-P203-UA/9EU);
- Vortex mixer;
- Vortex rotor for strips (using detection kit package S, strips REF R1-P203-S3/4EU);
- Tube rack for 1.5 mL tubes;
- PCR tube rack for 0.2 mL tubes or strips;
- 0.2 mL PCR tubes (using detection kit in the package U REF R1-P203-UA/9EU);
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- DTstream M1 dosage instrument (only for automated dosing using detection kit in the package U REF R1-P203-UA/9EU);
- Device for tray sealing DTpack ("DNA-Technology", LLC) (only for automated dosing using detection kit in the package U REF R1-P203-UA/9EU);
- Centrifuge for microtrays (only for automated dosing using detection kit in the package U REF R1-P203-UA/9EU);
- Polymer thermal seal for microtray sealing (only for automated dosing using detection kit in the package U REF R-P203-UA/9EU);
- PCR microtray (only for automated dosing using detection kit in the package U REF R1-P203-UA/9EU);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

Post-Amplification – Amplification detection area:

- Real-time PCR thermal cycler.

Software:

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

The OS supported: all versions of Windows starting from 7.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **HHV8 REAL-TIME PCR Detection Kit** except TechnoTaq MAX polymerase (package U) must be stored at temperatures from 2 °C to 8 °C over the storage period. TechnoTaq MAX polymerase must be stored at temperatures from minus 18 °C to minus 22 °C during the storage period.

PCR-mix must be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

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

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

Transportation of the kit, except the TechnoTaq MAX polymerase, is allowed in termobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 5 days and should be stored at temperatures from 2 °C to 8 °C immediately on receipt.

It is allowed to transport the TechnoTaq MAX polymerase in termobox with ice packs by all types of roofed transport at temperatures up to 25 °C but no more than 5 days and should be stored at temperatures from minus 18 °C to minus 22 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container:

- components of the kit should be stored at temperatures from 2 °C to 8 °C during the storage period;
- TechnoTaq MAX polymerase should be stored at temperatures from minus 18 °C to minus 22 °C during the storage period;
- PCR-mix for amplification should be stored at temperatures from 2 °C to 8 °C and out of light during the storage period.

The kit stored in under undue regime should not be used.

An expired the **HHV8 REAL-TIME PCR Detection Kit** should not be used.

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

The conformity of the **HHV8 REAL-TIME PCR Detection Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **HHV8 REAL-TIME PCR Detection Kit**.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All oligonucleotide components are produced by artificial synthesis technology according to internal quality control protocol and do not contain blood or products of blood processing.

Positive control is produced by artificial DNA synthesis technology. Positive control does not include parts of infectious agents.

All the liquid solutions are designed for single use and can not be used more than once in amplification

reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should 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 introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the 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. Amplification products must be handled in such a way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be exclusively employed for this specific purpose. Remove PCR waste only in a closed form. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Do not open the tubes after amplification. Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Inhalation: Inhalation of the PCR-mix contained within this kit is unlikely, however care should be taken.

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **HHV8 REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from blood, bioptate or punctate from foci of organ and tissue lesions.

Interfering substances

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

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

The maximum concentrations of interfering substances, that have no effect on the amplification of the laboratory control sample and internal control are: hemoglobin – 0.35 mg/mL of the DNA sample, isopropyl alcohol – 100 µL/mL of the DNA sample, methyl acetate – 100 µL/mL of the DNA sample.

Impurities contained in the biomaterial sample are almost completely removed during the DNA extraction. To reduce the count of PCR inhibitors, it is necessary to follow the principles of taking biological material. Suspecting a large count of PCR inhibitors in the sample, it is recommended to choose DNA extraction methods that allow to remove PCR inhibitors from the sample as much as possible. It is not recommended to use express methods of DNA extraction

General requirements

PCR analysis is a direct method, so taking of biological material must be carried out from the location of the infectious process. The decision about analyzing the location of sampling is done by a physician according to anamnesis and clinical picture.

The quality of taking a sample of biomaterial, its storage, transportation and pre-processing have a great importance for obtaining correct results.

Incorrect sample taking can lead to invalid results and the need for resampling.

ATTENTION! Before DNA extraction pre-processing of samples is needed.

Sample collection

ATTENTION! Pretreatment, sampling and storage of the material is carried out in accordance with the user manual for DNA extraction kit.

Peripheral blood

Peripheral blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of ethylenediaminetetraacetate (EDTA) at a final concentration of 2.0 mg/mL. The use of sodium citrate anticoagulant is also applicable. After taking the material, it is necessary to mix the blood with anticoagulant inverting the tube 2 – 3 times.

ATTENTION! It is not allowed to use heparin as an anticoagulant.

Bioptates

Bioptates are transferred to a 1.5 mL tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR. After sample collection the tube is tightly closed and marked.

Punctate

Collect the punctate (approximately 500 µL) in a sterile dish, close the cap of the tube tightly, and mark it.

Transportation and storage of the samples

Samples may be transported and stored 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 18 °C to minus 22 °C for one month.

In case of usage transport media biological material samples are transported and stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Sample preparation

Peripheral blood

The detailed description of peripheral blood preprocessing procedure is cited in extraction kits user manuals.

Bioptates

1. Vortex the tube with sample for 3-5 seconds, then spin down drops for 3-5 seconds.
2. Remove supernatant.

The following sample preparation is made according to user manual for the extraction kit used.

ATTENTION! When extracting DNA from biopsy specimens, only **PREP-NA** and **PREP-GS** extraction kits should be used.

Punctate

1 Transfer 500 µL of biomaterial into 1.5 mL tube.

2 Centrifuge the tube at RCF(g) 16000 for 10 minutes at room temperature (from 18 °C to 25 °C).

3 Remove the supernatant, leaving approximately 50 µL in the tube (precipitate + liquid fraction).

4 Add 500 µL of sterile physiological solution to the precipitate.

5 Vortex the tube for 3-5 seconds, then spin down drops for 3-5 seconds.

6 Centrifuge the tube at RCF(g) 16000 for 10 minutes.

7 Remove the supernatant, leaving the volume of precipitate + liquid fraction in the tube that is recommended in the instruction for the DNA extraction kit.

ATTENTION! When extracting DNA from biopsy specimens, only **PREP-NA** and **PREP-GS** extraction kits should be used.

ATTENTION! The detailed description of sampling and sample processing procedures as well as sample storage and transportation requirements are cited in **PREP-NA**, **PREP-GS, PREP-RAPID** and **PREP-MB RAPID** extraction kits user manuals.

8. PROCEDURE

DNA extracting from biological material

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

For DNA extraction from peripheral blood, it is allowed to use any reagent kits registered as a medical device and recommended by the manufacturers for DNA extraction from the corresponding types of biomaterial.

ATTENTION! Independently of DNA extraction kit used, a negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control in volumes as indicated.

Assay procedure

8.1 Preparing PCR for package S

ATTENTION! The reagents and tubes should be kept away from direct sun light.

ATTENTION! When using package S (R1-P203-S3/9EU), strips, strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips from the other kits!

8.1.1. Mark tubes with PCR-mix for each test sample, negative control (C-) and positive control (C+).

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

8.1.2. Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.1.3. Add 10 µL of Taq-polymerase solution into each tube. Avoid paraffin layer break.

8.1.4. Add one drop (~20 µL) of mineral oil into each tube (not applicable to kits approved for use with Rotor-Gene thermal cycler). Close the tubes.

8.1.5. Vortex the tubes with samples, "C-" and "C+" for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

In case of using **PREP-MB RAPID DNA Extraction Kit**, after vortexing put the tubes with the DNA preparation in magnetic rack. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

ATTENTION! Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next DNA sample to prevent contamination. In case of using tubes in strips, close the strip before proceeding to the next DNA sample to prevent contamination. Close the tubes/strips tightly. Use filter tips.

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

8.1.7. Add 5.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.1.8. Spin tubes/strips for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

8.1.9. Set the tubes/strips into the Real-time Thermal Cycler.

8.1.10. Launch the operating software for DT instrument[1]. Add corresponding test[2], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 8.1.9) and run PCR. See Table 5.

For use with Rotor-Gene Q real-time thermal cycler consult user manual for devices. See Tables 6.

[1] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[2] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 5. The PCR program for DTlite and DTprime Thermal Cyclers

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		v	
3	94	0	10	45		Cycle
	64	0	15		v	
4	94	0	5	1		Cycle
5	10	…	…	Holding		Holding

Table 6. The PCR program for Rotor-Gene Q thermal cycler

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	90 sec	
Cycling	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 2	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement.			

8.2 Preparing PCR for package U, manual dosing

ATTENTION! The reagents and tubes should be kept away from direct sun light.

8.2.1 Mark the required number of 0.2 mL tubes for each test sample, positive control (C+) and negative control (C-).

Example: to test 4 samples, mark 4 tubes for samples, 1 tube for “C-” and 1 tube for “C+”. The resulting number of tubes is 6.

8.2.2 Vortex the tube with PCR-mix for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

8.2.3 Add to each tube 6.0 µL of PCR-mix.

8.2.4 Vortex the TechnoTaq MAX polymerase and PCR buffer for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

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

8.2.5 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase. Add into the one tube:
6.0 x (N+1) µL of PCR-buffer,
0.3 x (N+1) µL of TechnoTaq MAX polymerase,
N is a quantity of the samples to be tested taking to account “C-”, “C+”.

Example: for simultaneous testing of 4 samples, “C-” and “C+” in one PCR run, mark 6 tubes (4 tubes for samples to be tested, 1 tube for “C+” and 1 tube for “C-”). Prepare the mixture of PCR-buffer and Taq-polymerase for 7 (6+1) tubes. Mix 42 µL of PCR-buffer and 2.1 µL of TechnoTaq MAX polymerase.

8.2.6 Vortex the tube with the mixture of PCR-buffer and TechnoTaq MAX polymerase for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

ATTENTION! Mixture of PCR-buffer and TechnoTaq MAX polymerase must be prepared immediately prior to use.

8.2.7 Add 6.0 µL of PCR-buffer and TechnoTaq MAX polymerase mixture into each tube with PCR-mix.

ATTENTION! Follow the steps listed in pp. 8.2.8 – 8.2.13 within two hours after addition of PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.2.8 Vortex the tubes with samples, “C+” and “C-“ for 3-5 seconds and spin down drops for 1-3 seconds.

ATTENTION! In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

In case of using **PREP-MB RAPID DNA Extraction Kit**, after vortexing put the tubes with the DNA preparation in magnetic rack. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

ATTENTION! Open the cap of the tube, add DNA sample (or control sample), then close the tube before proceeding to the next tube to prevent contamination. Close the tubes tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding tubes. Do not add DNA into the “C+”, “C-” tubes.

8.2.10 Add 6.0 µL of negative control (C-) which passed whole DNA extraction procedure into corresponding tube. Add 6.0 µL of positive control sample (C+) into corresponding tube.

8.2.11 Spin tubes for 3-5 seconds (when using the Rotor-Gene Q thermal cycler, centrifugation is not required).

8.2.12 Set the tubes into the Real-time Thermal Cycler.

8.2.13 Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and ID’s of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 8.2.12) and run PCR. See Table 7.

Amplification program for Rotor-Gene Q thermocycler is contained in the Table 8.

[3] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.

[4] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

Table 7. The PCR program for DTlite and DTprime Thermal Cyclers for 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	10			Holding		Holding

Table 8. The PCR program for Rotor-Gene Q thermal cycler for package U

Cycling	Temperature	Hold time	Cycle repeats
Cycling	80 deg	60 sec	1 time
	94 deg	300 sec	
Cycling2	94 deg	30 sec	5 times
	57 deg*	15 sec	
Cycling 2	94 deg	10 sec	45 times
	57 deg*	15 sec	
* Take the measurement			

8.3 Preparing PCR using DTStream (only for package U)

ATTENTION! The reagents and tubes should be kept away from direct sun light.

8.3.1 Vortex the tube with PCR-mix for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

8.3.2 Vortex the TechnoTaq MAX polymerase and PCR buffer for 3-5 seconds, then spin in vortex for 1-3 seconds to collect the drops.

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

8.3.3 Prepare the mixture of PCR buffer with TechnoTaq MAX polymerase according to the user manual for dosing device DTstream.

8.3.4 Vortex the tube with the mixture for 3-5 seconds, the spin in vortex for 1-3 seconds to collect the drops.

8.3.5 Vortex the tubes with DNA samples, “C-” and “C+” for 3-5 seconds and spin down the drops in vortex for 1-3 seconds.

ATTENTION! In case of using **PREP-GS DNA Extraction Kit**. After vortexing centrifuge the tubes with the DNA preparation at RCF(g) 16000 for one minute to precipitate the sorbent. If, after isolation, the supernatant containing the isolated DNA was transferred to new tubes, centrifugation is carried out for 1-3 seconds in a vortex mixer.

In case of using **PREP-MB RAPID DNA Extraction Kit**, vortex the tubes for 3-5 seconds on a vortex mixer, put the tubes with the DNA preparation in magnetic rack and transfer the supernatant containing the isolated DNA to new tubes. If, after DNA extraction, the supernatant containing the isolated DNA was already transferred to new tubes, centrifugation is carried out for 3-5 seconds in a vortex mixer.

8.3.6 Put the tubes with PCR-mix, the mixture of PCR-buffer and TechnoTaq MAX polymerase, DNA samples, positive and negative controls and PCR microtray on the DTstream working table and conduct dosage of the components according to DTstream user manual.

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

8.3.8 Run the process of sealing of PCR microtray according to the user manual of DTpack sealing device.

8.3.9 Centrifuge the microtray on RCF(g) 100 for 30 seconds.

8.3.10 Put the PCR microtray into the thermoblock of detecting thermocycler.

8.3.11 Launch the operating software for DT instrument[5]. Add corresponding test[6], specify the number and ID's of the samples, positive and negative control samples. Specify the position of the tubes/strips in the thermal unit (see 8.3.10) and run PCR. See Table 7.

9. CONTROLS

The **HHV8 REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the HHV8 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.

ATTENTION! A negative control sample should go through all stages of DNA extraction. Physiological saline solution or negative control sample from an extraction kit can be used as a negative control in volumes as instructions.

For **HHV8 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 **HHV8 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.

[5] Please, apply to Operation Manual for DTprime and DTlite Real-Time PCR instruments PART II.
[6] Instructions for uploading "files with test parameters" can be found on "DNA-Technology's" website https://www.dna-technology.com/assaylibrary.

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

In case of using DNA-Technology made Real-Time PCR Thermal Cyclers, the analysis is performed automatically. In all other cases, the analysis is based on the presence or absence of specific signal.

In the samples containing HHV8 DNA (specific product), the detecting amplifier registers the expressed growing fluorescence of specific product, the amplification result of the internal control is not taken into account.

In the samples free of HHV8 DNA, the detecting amplifier registers the expressed growing fluorescence of the internal control and its absence for the specific product.

When the unseen expressed growing fluorescence or negative result of both in the specific product and the internal control, the result of amplification is considered as uncertain. It may due to inhibitors, incorrect performance, non-compliance of the amplification temperatures, etc. In this case, amplification, or DNA extraction, or collecting of clinical material are required to be repeated.

In case the result for negative control is defined as positive, the whole experiment should be considered false. The retesting and decontamination are required.

11. SPECIFICATIONS

a. The analytical **specificity** of the **HHV8 REAL-TIME PCR Detection Kit** was assessed by bioinformatics analysis using available on-line databases with up-to-date comprehensive genetic information. The specific oligonucleotides used in the test were checked against GenBank database sequences. None of the sequences showed sufficient similarity for unspecific detection.

The samples with HHV8 DNA are to be registered positive for specific product (a fragment of the HHV8 genome). The samples free of HHV8 DNA are to be registered negative for specific product and positive for internal control.

No nonspecific positive amplification results were shown in the presence of Cytomegalovirus, Herpes symplex virus 1,2, Epstein-Barr virus, Human herpes virus 6, Varicella-Zoster virus, HPV6, HPV 11 DNA, and human DNA at a concentration of up to 1.0×10^8 copies/mL of the sample.

b. The analytical **sensitivity** is 5 copies of Human Herpes virus type 8 DNA per amplification tube.

The analytical sensitivity was set by analyzing serial dilutions of a laboratory control (LC). A total of 94 runs were made for each concentration.

LC concentration, copies per amplification tube	Number of runs	Number of positive results	% of positive results
5	94	94	100
2	94	88	93.6
0.5	94	52	55.3
0	94	0	0

Note. The analytical sensitivity for DNA in the sample depends on the sample preparation method and the final volume of extracted DNA (elution volume).

E x a m p l e : The analytical sensitivity of 5 DNA copies per amplification tube corresponds to the following values of DNA concentration when using nucleic acid extraction kits manufactured by “DNA-Technology R&P”, LLC:

Sample	**Nucleic acids extraction kits**			
	PREP-NA	**PREP-GS**	**PREP-MB RAPID (at elution of 300 µL)**	**PREP-RAPID**
- punctate (when extracted from 500 µL sample); - bioptates*	50 copies/sample	100 copies/sample	300 copies/ sample	500 copies/sample
* Only **PREP-GS** and **PREP-NA** extraction kits are used for DNA extraction from bioptates				

ATTENTION! The claimed specifications are guaranteed when DNA extraction is performed with **PREP-NA** REF P-002/1EU, **PREP-GS** REF P-003/1EU, **PREP-RAPID** REF P-001/1EU and **PREP-MB RAPID** REF P-116-N/4EU, P-116-A/8EU 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

"DNA-Technology Research&Production", LLC declares that the abovementioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our official representative in EU by quality issues of **HHV8 REAL-TIME PCR Detection Kit.**

Technical support:

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14. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Date of manufacture
	Temperature limit		Consult instructions for use
Σ	Contains sufficient for <n> tests	REF	Catalogue number
	Use-by date		Manufacturer
LOT	Batch code		Keep away from sunlight
VER	Version	CONTROL +	Positive control
NON STERILE	Non-sterile		Caution
EC REP	Authorized representative in the European Community		

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