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RUO

**For research use only**

# HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit

# INSTRUCTION FOR USE

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R1-P211-23/9EU
R1-P211-UA/9EU

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## 1. INTENDED USE

The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** is intended for detection of *Herpes simplex virus 1* (HSV1), *Herpes simplex virus 2* (HSV2) and *Cytomegalovirus* (CMV) DNA in human biological material (urine, scrapes of epithelial cells from the urogenital tract) by real-time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit.**

The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** can be used in research practice.

Potential users: qualified personnel trained in molecular research methods and rules of work in the laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

## 2. METHOD

Method: polymerase chain reaction (PCR) with detection of the results in real time; multiplex qualitative analysis.

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. For package S, “hot” start is provided by reaction mixture preparation consisting of two layers separated by a layer of paraffin. The polymerase chain reaction starts only when paraffin is melted. “Hot” start for package U is provided by using polymerase whose activity is blocked by antibodies, the activation of the enzyme occurs only after preheating the reaction mixture at 94 °C. This eliminates the nonspecific binding of primers to the DNA target during the initial heating of the test tube.

DNA probes, each containing a fluorescent label and a fluorescence quencher, are introduced into the PCR-mix. When a specific product is formed, the DNA probe is destroyed and the effect of the quencher on the fluorescent label stops, which leads to an increase in the fluorescence level recorded by detecting thermal cycler. The number of destroyed probes (and therefore the fluorescence level) increases in proportion to the number of specific amplicons produced. The fluorescence level is measured at each amplification cycle in real time.

The PCR-mix includes the internal control (IC), which is intended to assess the quality of the polymerase chain reaction.

The DNA probe used to detect the *Herpes simplex virus 2* (HSV2) amplification product DNA includes the fluorescent dye Fam. The DNA probe used to detect *Cytomegalovirus* (CMV) amplification product DNA includes the fluorescent dye Rox. The DNA probe used to detect *Herpes simplex virus 1* (HSV1) amplification product DNA includes the fluorescent dye Cy5. The DNA probes used to detect the amplification product of an internal control (IC) include the fluorescent dye Hex.

Using several fluorescent dyes allows to reduce the number of tubes and biomaterial required for the test, because there is a possibility to register results of different amplification reactions in one tube simultaneously.

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 |
|---|---|---|---|---|
| *Herpes simplex virus 2* (**HSV2**) | IC | *Cytomegalovirus* (**CMV**) | *Herpes simplex virus 1* (**HSV1**) | - |

The automatic analysis is available on "DNA-Technology" made instruments DTlite or DTprime real-time thermal cyclers for **HSV1/HSV2/CMV Multiplex 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 **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** is also approved for use with Rotor-Gene Q (QIAGEN), CFX96 (Bio-Rad) and Applied Biosystems Quant Studio 5 (Life Technologies Holdings Pte. Ltd) real-time thermal cyclers.

## 3. CONTENT

The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** content is represented in Tables 2-4.

Table 2. The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P211-S3/9EU

| Reagent | Description | Total volume | Amount |
|---|---|---|---|
| Paraffin sealed PCR-mix | Colorless or pink transparent liquid under waxy white fraction | 1,920 µL (20 µL in each) | tubes, 12 strips of 8 |
| Taq-polymerase solution | Colorless transparent liquid | 1,000 µL (500 µL in each) | 2 tubes |
| Mineral oil | Colorless transparent viscous oily liquid | 2.0 mL (1.0 mL in each) | 2 tubes |
| Positive control[1] | Colorless transparent liquid | 130 µL | 1 tube |
| Strip caps | 12 strips of 8 | | |

Table 3. The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P211-23/9EU

| Reagent | Description | Total volume | Amount |
|---|---|---|---|
| Paraffin sealed PCR-mix | Colorless or pink transparent liquid under waxy white fraction | 1,920 µL (20 µL in each) | 96 individual tubes |
| Taq-polymerase solution | Colorless transparent liquid | 1,000 µL (500 µL in each) | 2 tubes |
| Mineral oil | Colorless transparent viscous oily liquid | 2.0 mL (1.0 mL in each) | 2 tubes |
| Positive control[1] | Colorless transparent liquid | 130 µL | 1 tube |

[1] - marking as C+ is allowed

Table 4. The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** content, package U, tubes for R1-P211-UA/9EU

| Reagent | Description | Total volume | Amount |
|---|---|---|---|
| PCR-mix | Colorless or pink transparent liquid | 600 µL | 1 tube |
| 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 |

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

The reagent kit in package S is intended for single use and designed for 96 tests (including one negative control and one positive control in each run). It is recommended to perform no more than 24 runs.

The reagent kit in package U is designed for 96 tests given that there are at least 5 samples per run (3 test samples, negative control and positive control).

## 4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

### 4.1. Specimen collection

- Sterile single-use swabs, sterile single-use flasks and sterile containers to collect clinical material;
- Sterile tubes containing transport media: "DNA-Technology" made **PREP-RAPID** ( REF P-001/1EU) (not applicable to male urethral smears), **STOR-F** ( REF P-901-1/1EU, REF P-901-N/1EU, P-901-R/1EU), **STOP-M** ( REF P-910-1/1EU) or equivalent or sterile physiological saline solution for the transportation of the sample.

### 4.2. DNA extraction and PCR

Preamplification-specimen and control preparation area:

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) no less than 16,000);
- Solid-state thermostat (temperature range 50-65 °C);
- 1.5 mL tubes;
- Tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 20-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL; 200 µL; 1,000 µL);
- Pipette stand;
- Nucleic acid extraction kit ("DNA-Technology" made **PREP-RAPID** ( REF P-001/1EU) (not applicable to male urethral smears), **PREP-NA** ( REF P-002/1EU), , **PREP-NA PLUS** ( REF P-002/2EU), **PREP-GS** ( REF P-003/1EU), **PREP-GS PLUS** ( REF P-003/2EU) **PREP-OPTIMA**

( REF P-016-1/2EU, REF P-016-N/2EU), **PREP-MB RAPID** ( REF P-116-N/4EU, REF P-116-A/8EU), and **PREP-MB-RAPID II** ( REF P-122-P/9EU, REF P-124-P/9EU) extraction kits are recommended);

- Transport medium (if necessary) ("DNA-Technology" made **STOR-F** ( REF P-901-1/1EU, REF P-901-R/1EU) or **STOR-M** ( REF P-911-1/1EU) 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 with freezer;
- Vortex mixer;
- Vortex rotor for 0.2 mL strips (in case of using package S, strips);
- PCR tube rack for 0.2 mL strips;
- tube rack for 0.2 mL tubes;
- tube rack for 1.5 mL tubes;
- Single channel pipettes (dispensers covering 0.5-1,000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL; 20 µL; 200 µL; 1,000 µL);
- Pipette stand;
- RNase and DNase free 1.5 mL microfuge tubes with caps;
- RNase and DNase free 0.2 mL PCR tubes or 96-well microplate (cannot be used with DTlite thermal cycler);
- DTstream 12M1 or 15M1 dosing device ("DNA-Technology", LLC) (only for automated dosing in case of using package U);
- RNase and DNase free filtered pipette tips (volume 200 µL) for DTstream dosing device or similar (only for automated dosing in case of using package U);
- Device for plate sealing DTpack ("DNA-Technology", LLC) (only for automated dosing in case of using package U);
- Centrifuge for microplates (RCF(g) at least 100) (only for automated dosing in case of using package U);
- Polymer thermal film for microplate sealing (in case of using package U);
- 384-well microplate (only for automated dosing in case of using package U);
- 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. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

### 5.1. Transport and storage conditions. Package S

- All components of the reagent kit must be stored in a refrigerator at the temperature from 2 °C to 8 °C throughout the shelf life of the kit.
- Paraffin sealed PCR-mix must be kept away from light.
- Transport 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 components included in the kit.
- It is allowed to transport the kit in thermoboxes with ice packs by all types of roofed transport at the temperature inside the container from 2 °C to 25 °C for no longer than 5 days.

### 5.2. Transport and storage conditions. Package U

- All components of the reagent kit, except for TechnoTaq MAX polymerase, must be stored in a refrigerator or a cooling chamber at the temperature from 2 °C to 8 °C throughout the shelf life of the kit.
- PCR-mix must be kept away from light.
- TechnoTaq MAX polymerase must be stored in a freezer at the temperature from minus 22 °C to minus 18 °C throughout the shelf life of the kit.
- 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 components included in the kit.
- 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 container 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 container 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 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 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 **HSV1/HSV2/CMV Multiplex 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.

The conformity of the **HSV1/HSV2/CMV Multiplex 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

Only personnel trained in the methods of molecular research are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the analysis 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 analysis. 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 transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When 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 **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** is designed to detect DNA extracted from urine and scrapes of epithelial cells from the urogenital tract.

### 7.1. Interfering substances

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

The following substances are considered to be PCR inhibitors that may be present in the DNA sample: Hemoglobin and pharmaceuticals present in the DNA sample as a result of incomplete removal during DNA extraction from the biomaterial sample, and isopropyl alcohol and methyl acetate remaining in the DNA sample as a result of incomplete removal of wash solutions during sample preparation.

The maximum concentrations of interfering substances, that have no effect on the amplification of the laboratory control 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.

To assess the possible interference of drugs, we selected those potentially present in residual amounts in human biological samples taken from the corresponding biotopes under analysis (Miramistin®, chlorhexidine bigluconate).

For all the drugs under analysis no effect was found in concentration up to 10% in biomaterial sample.

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

In case biomaterial from several biotopes is needed, repeat the procedure taking each sample into a new

tube.

Incorrect sampling may lead to invalid results and the need for resampling.

Use RNase and DNase free filter tips at biomaterial preparation stage.

To prevent contamination, only open the cap of the tube where the sample is to be introduced, and close it before proceeding to the next one.

### 7.3. Sample collection

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

**WARNING!** Pretreatment, sampling and storage of the material is carried out in accordance with the instruction for use for DNA extraction kit.

#### 7.3.1. Urine

Sampling is performed in accordance with the NA reagent kit instruction for use.

#### 7.3.2. Scrapes from urogenital tracts

**WARNING!** Sample intake into the tubes with the **PREP-RAPID** reagent must be done with a dry swab! Avoid contact of solution with skin, eyes and mucous membranes.
**Method limitations[2]:** local application of medicines, vaginal ultrasound, colposcopy less than 24 hours before the test.
Sampling is performed in accordance with the NA reagent kit instruction for use.

**7.3.2.1** Urogenital scrapes sampling

Women on the day before the examination should not carry out genital toilet and sprays. In order to obtain an accurate result, it is necessary that the material to be examined contains as many epithelial cells as possible and a minimum amount of mucus and blood admixture.
**WARNING!** Before obtaining an epithelial scrape from the urethra, posterior vaginal vault and cervical canal, the free-flowing secretion should be removed with a sterile cotton swab.
If it is necessary to take biomaterial from several biotopes, repeat the procedure, each time taking the material with a new probe into a new tube.

**7.3.2.2** Vaginal sampling

The material must be taken before manual examination. The mirror before manipulation can be moistened with hot water, the use of antiseptics to treat the mirror is contraindicated. Scrape is taken from the posterolateral vaginal vault. In girls, the material is taken from the mucous membrane of the vaginal vestibule, and in some cases from the posterior vaginal vault through the hymenal rings.

**7.3.2.3** Urethral sampling

It is recommended to abstain from urinating for 1.5-2 hours before biomaterial sampling.
Immediately prior to sampling treat the external opening of the urethra with a tampon, which can be moistened with sterile saline solution.
In the presence of purulent discharge it is recommended to take a scrape 15-20 minutes after urination, in the absence of discharge it is necessary to massage the urethra with a probe to take biomaterial. In women, the probe is inserted into the urethra to a depth of 1.0-1.5 cm, in children, the material for the analysis is taken only from the external opening of the urethra.

**7.3.2.4** Cervical canal sampling

[2] - if it does not contradict the requirements to the NA extraction reagent kits being used

Before sampling remove mucus with a cotton swab and then treat the cervix with sterile physiologic solution. The probe is inserted into the cervical canal to a depth of 0.5-1.5 cm. When removing the probe, it is necessary to completely exclude its touching the vaginal walls.

### 7.3.3. Transport and storage of samples

**Urine**

Urine samples transport and storage conditions are determined by the NA extraction reagent kit instruction or instruction to the transport medium used for sample transport and storage.

Urine samples must be transported and stored (if it does not contradict the requirements for the NA extraction reagent kit):

- at temperature from 2 °C to 8 °C for no longer than 1 day;
- at temperature from minus 22 °C to minus 18 °C for no longer than one week.

**WARNING!** Only one freezing-thawing of material is allowed.

**Scrapes from urogenital tract**

Transport and storage conditions for scrapes from urogenital tract are determined by the instructions for the transport media used for transportation and storage of the samples or by instructions for the recommended NA extraction kits.

It is allowed to store samples at temperature from 2 °C to 8 °C for no longer than 24 hours. If it is not possible to deliver samples to lab throughout the day, one-time freezing is allowed. It is allowed to store frozen samples at temperature from minus 22 °C to minus 18 °C for one month (if it does not contradict the requirements to the NA extraction reagent kit).

**WARNING!** Only one freezing-thawing of material is allowed.

**Sample preparation**

Sample preparation (if necessary) is performed in accordance with the NA reagent kit instruction for use.

## 8. PROCEDURE

**DNA extraction from biological material**

DNA extraction is carried out in accordance with the instruction to the extraction kit. **PREP-RAPID**, **PREP-NA, PREP-NA PLUS**, **PREP-GS**, **PREP-GS PLUS, PREP-OPTIMA**, **PREP-MB RAPID** and **PREP-MB-RAPID II** extraction kits are recommended.

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

**Analysis procedure**

### 8.1. PCR preparation for package S

**WARNING!**

1. The reagents and tubes should be kept away from direct sun light.
2. Strictly observe the completeness of the strips and caps for them. Do not use the caps for the strips of the other kits!

8.1.1 Mark one tube/stripped tube with the paraffin sealed PCR-mix for each test sample, negative control (C-), positive control (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 negative control tube "C-" and one positive control tube "C+". Total number of tubes is 6.

8.1.2 Shake the tubes with Taq-polymerase solution on vortex mixer for 3-5 seconds and spin in a vortex mixer for 1-3 seconds.

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

**WARNING!** When using the Rotor-Gene Q detecting thermal cycler for PCR, no mineral oil is added to the tubes!

8.1.4 Add one drop (~20 µL) of mineral oil into each strip tube. Cover the tubes/strips loosely with caps.

8.1.5 Shake the tube with positive control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

**WARNING!**

1. Before introducing DNA preparation and negative control into tubes with PCR-mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-RAPID**, **PREP-NA** and **PREP-NA PLUS,** and **PREP-GS** and **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB RAPID** DNA extraction kit, it is necessary to carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. If strips are used, close the strip caps after adding the sample before proceeding with the next sample. 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 tubes "C-", and "C+". Avoid paraffin layer break.

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

8.1.8 Add 5.0 µL of positive control sample (C+) into corresponding tube. Avoid paraffin layer break.

8.1.9 Spin the tubes/strips for 3-5 seconds in a vortex mixer (if using Rotor-Gene Q thermal cycler, spinning is not required).

8.1.10 Set the strips into the real-time thermal cycler.

8.1.11 Launch the operating software for DT instrument[3]. Add corresponding test[4], specify the number and IDs of the samples, positive and negative controls. Specify the position of the strips in the thermal unit and run PCR. See Table 5.

8.1.12 For CFX96, Applied Biosystems QuantStudio 5 and Rotor-Gene Q detecting thermal cyclers: perform PCR considering reaction mixture volume of 35 µL according to amplification programs shown in Tables 6- 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 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 | | √ | |
| | | | | | | |
| 3 | 94 | 0 | 10 | 45 | | Cycle |
| | 64 | 0 | 15 | | √ | |
| | | | | | | |
| 4 | 94 | 0 | 5 | 1 | | Cycle |
| | | | | | | |
| 5 | 25[5] | | … | Holding | | Holding |
| √ - optical measurement | | | | | | |

Table 6. The PCR program for CFX96 thermal cyclers (packages S, U)

| Step | Temperature, °C | Time min: sec | Number of cycles (repeats) |
|---|---|---|---|
| 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, Rox and Cy5 channels at 64 °C | | | |

Table 7. The PCR program for Applied Biosystems QuantStudio 5 thermal cyclers (packages S, U)

| Stage | Step | Temperature, °C | Time min: sec | Number of cycles (repeats) |
|---|---|---|---|---|
| Holding | 1 | 80 | 01:00 | 1 |
| | 2 | 94 | 01:30 | 1 |
| PCR | 1 | 94 | 0:20 | 50 |
| | 2 | 62 **√** | 0:20 | |
| √- data collection for fluorophores (Fam, Vic (Hex), Rox, Cy5) is on | | | | |

Table 8. The PCR program for Rotor-Gene Q thermal cyclers (package S, tubes)

| Cycling | Temperature | Hold Time | Cycle Repeats |
|---|---|---|---|
| Cycling 1 | 80 deg | 60 sec | 1 time |
| | 94 deg | 90 sec | |
| Cycling 2 | 94 deg | 30 sec | 5 times |
| | 62 deg √ | 15 sec | |
| Cycling 3 | 94 deg | 10 sec | 45 times |
| | 62 deg √ | 15 sec | |
| √– optical measurements, set Acquiring on Green, Yellow, Orange and Red detection channels at 62 °C. | | | |

[5] Holding at 10 °C is allowed

### 8.2. PCR preparation for package U, manual dosing

**WARNING!**

1. For amplification use 0.2 mL single-use amplification tubes or 96-well PCR microplates[6], sealed hermetically with thermal film. It is not recommended to use strips due to postamplification contamination hazard.
2. The reagents and tubes should be kept away from direct sunlight.

8.2.1 Mark the required number of 0.2 mL tubes or a 96-well PCR microplate for each test sample, negative control (C-) and positive control (C+).

Note: It is recommended to test at least 5 samples per test (3 test samples, negative control and positive control).

**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.

8.2.2 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 6.0 µL of PCR-mix to each tube/well (including "C-" and "C+").

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

**WARNING!** Take TechnoTaq MAX polymerase 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,
where N is the number of marked tubes/microplate wells considering "C-", "C+".

**Example:** to test 4 samples, "C-" and "C+" (6 in total) in one PCR run, prepare the mixture of PCR-buffer and Taq-polymerase for 7 (6+1) tubes/wells, i.e. 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 on vortex for 1-3 seconds to collect the drops.

**WARNING!** 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/well with PCR-mix. Cover the tubes loosely.

**WARNING!** Follow the steps 8.2.8 – 8.2.14 within two hours after adding PCR-buffer and TechnoTaq MAX polymerase mixture to PCR-mix.

8.2.8 Vortex the tube with positive control "C+" for 3-5 seconds and spin down the drops for 1-3 seconds.

**WARNING!**

1. Before introducing DNA preparation and negative control into tubes with PCR-mix, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-RAPID**, **PREP-NA** and **PREP-NA PLUS,** and **PREP-GS** and **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.

[6] - 96-well plates are not used with DTlite detecting thermal cycler

3. In case of using **PREP-MB RAPID** DNA extraction kit, it is necessary to carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control "C-" on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.
4. To prevent contamination, only open the caps of the tubes into which the sample is to be added and close them before adding the next sample. Close the tubes/strips tightly. Use filter tips.

8.2.9 Add 6.0 µL of DNA sample into corresponding tubes/wells. Do not add DNA into the "C-", "C+" tubes/wells.

8.2.10 Add 6.0 µL of negative control ("C-") which passed whole DNA extraction procedure into the corresponding tube/well.

8.2.11 Add 6.0 µL of positive control sample ("C+") into the corresponding tube/well.

8.2.12 In case of using 96-well PCR microplates:

8.2.12.1. Place the PCR microplate carefully, without shaking into the DTpack sealing device.

8.2.12.2. Seal the PCR microplate with polymer thermal film according to the DTpack operation manual.

8.2.12.3. Centrifuge the plate at RCF(g) 100 for 30 seconds.

8.2.13 In case of using tubes:
Spin the tubes for 3-5 seconds on vortex mixer.

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

8.2.15 Launch the operating software for DT instrument[7]. Add corresponding test[8], specify the number and IDs of the samples, positive and negative controls. Specify position of the tubes in thermal unit (see 8.2.14) and run PCR. See Table 9.

8.2.16 For CFX96 and Applied Biosystems QuantStudio 5 thermal cyclers perform PCR considering the volume of reaction mixture of 18 µL. See Tables 6, 7.

Table 9. 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 | 05 | 15 | | Cycle |
| | 94 | 0 | 05 | | | |
| | | | | | | |
| 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* | | | Holding | | Holding |
| √ - optical measurements<br>* - holding at 10 °C is allowed | | | | | | |

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

### 8.3. PCR preparation for package U, using DTstream

**WARNING!**

1. For amplification use 384-well microplates hermetically sealed with thermal film.
2. The reagents and tubes should be kept away from direct sun light.

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

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

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

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

8.3.3 Prepare the mixture of PCR-buffer and TechnoTaq MAX polymerase according to the software for DTstream.

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

8.3.5 Vortex the tubes with positive control for 3-5 seconds and spin in vortex for 1-3 seconds to collect the drops.

**WARNING!**

1. Before dosing, fulfill the recommendations for DNA preparation use listed in the NA extraction reagent kit instruction for use.
2. In case of using **PREP-RAPID**, **PREP-NA** and **PREP-NA PLUS,** and **PREP-GS** and **PREP-GS PLUS** extraction kits (only if supernatant containing the extracted DNA was transferred to new tubes after extraction), shake the tubes with DNA preparation and negative control for 3-5 seconds on vortex mixer and spin down the drops for 1-3 seconds.
3. In case of using **PREP-MB RAPID** DNA extraction kit, it is necessary to carefully, without shaking, centrifuge the tubes with DNA preparation and negative control on vortex for 1-3 seconds, then place the tubes into magnetic rack. If, after extraction, the supernatant containing the extracted DNA has been transferred to new tubes, shake the tubes with the DNA preparation and negative control “C-“ on vortex for 3-5 seconds and spin down the drops for 1-3 seconds.

8.3.6 Set the tubes with PCR-mix, the mixture 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.

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

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

8.3.9 Centrifuge the microplate at RCF(g) 100 for 30 seconds.

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

8.3.11 Launch the operating software for DT instrument[9]. Add corresponding test[10], specify the number and ID’s of the samples, positive and negative controls. Specify position of the tubes in thermal

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

unit and run PCR. See Table 9.

## 9. CONTROLS

The **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit** contains positive control. 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 solution or negative control sample from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

For **HSV1/HSV2/CMV Multiplex 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 considered;
- the exponential growth of the fluorescence level is absent for the specific product and is present for internal control.

For **HSV1/HSV2/CMV Multiplex 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 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** For CFX96 (Bio-Rad) detecting thermal cyclers, it is recommended to use the regression type of analysis (Cq Determination Mode: Regression) and select “Baseline Subtraction Curve Fit” in the tab “Baseline Subtraction”.

**10.3** The results are interpreted according to Table 10. The run results are valid if the conditions for interpreting the results obtained for the controls are fulfilled.

Table 10. PCR results interpretation

| **Detection channel** | | | | **Result interpretation** |
|---|---|---|---|---|
| Fam/Green, Cp/Cq/Ct | Hex/Yellow/Vic, Cp/Cq/Ct | Rox/Orange, Cp/Cq/Ct | Cy5/Red, Cp/Cq/Ct | |
| **Test samples** | | | | |
| **Specified** | Not considered | Not specified | Not specified | ***Herpes simplex virus 2*** **DNA is detected** |
| Not specified | Not considered | **Specified** | Not specified | ***Cytomegalovirus*** **DNA is detected** |
| Not specified | Not considered | Not specified | **Specified** | ***Herpes simplex virus 1*** **DNA is detected** |
| Not specified | **Specified** | Not specified | Not specified | DNA of the sought microorganisms is not detected |
| Not specified | Not specified | Not specified | Not specified | Unreliable result |
| **Negative control** | | | | |
| Not specified | **Specified** | Not specified | Not specified | **Negative result** The results are valid |
| **Positive control** | | | | |
| **Specified** | **Specified** | **Specified** | **Specified** | **Positive result** The results are valid |

**10.4** An invalid result may be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect performance of the analysis protocol; noncompliance with the amplification temperature regime, etc. In this case, PCR with the available DNA preparation needs to be repeated, or the DNA must be extracted and PCR performed again, or the biological material must be taken again (performed sequentially).

**10.5** Cp/Cq/Ct values obtained for the biological sample on Fam, Rox or Cy5 detection channels less than 24 indicate high concentration of the corresponding microorganism DNA. In this case a false-negative result is possible for the microorganism whose DNA is present in low concentration. To eliminate the possibility of false-negative results, we recommend to repeat PCR of the extracted DNA preparation using "DNA-Technology" made **Herpes simplex virus 1, 2 REAL-TIME PCR Detection Kit** and **CMV REAL-TIME PCR Detection Kit.**

**10.6** If positive result is obtained for negative control, the results of the whole run are considered unreliable. In this case, special measures for identification and elimination of possible contamination are required.

**10.7** If negative result is obtained for positive control, the results of the whole run are considered unreliable. In this case, a repeated amplification of the whole batch of samples is required.

## 11. SPECIFICATIONS

**a.** Analytical specificity

For human biomaterial samples containing the DNA of the detected microorganisms, the detecting thermal cycler software registers positive amplification results for the specific products (*Herpes simplex virus 1, Herpes simplex virus 2, Cytomegalovirus* genome fragments) on the corresponding detection channel.

For human biomaterial samples not containing the DNA of *Herpes simplex virus 1, Herpes simplex virus 2* or *Cytomegalovirus*, the detecting thermal cycler software registers negative amplification results for the specific product on the corresponding detection channels and positive result for the internal control (IC) on Hex/Yellow/Vic detection channel.

**ATTENTION!** If the initial DNA concentration of one of the sought microorganisms is high, the false negative results is possible for the microorganism whose DNA concentration is low (see p.10. DATA ANALYSIS).

The absence of nonspecific positive amplification results has been shown in high DNA concentrations of closely related microorganisms or microorganisms potentially present in the test samples: *Varicella-zoster virus, Epstein-Barr virus, Human herpesvirus 6, Human herpesvirus 7, Human herpesvirus 8, Staphylococcus spp., Streptococcus spp., Parvovirus B19, Chlamydia pneumonia, Mycoplasma hominis, Trichomonas vaginalis, Neisseria gonorrhoeae, Toxoplasma gondii, Mycoplasma genitalium, Chlamydia trachomatis, Gardnerella vaginalis* and human DNA in concentration up to $1.0\times10^8$ copies/mL of the sample.

**b.** Analytical sensitivity

Limit of detection amounts to 5 copies of each microorganism DNA per amplification tube.

Sensitivity is determined by the analysis of serial dilutions of two batches of the laboratory control sample (LCS).

Limit of detection in test sample depends on the used DNA extraction kit and the amount of the extracted DNA preparation obtained:

| Biomaterial | Kits for DNA extraction | Volume of obtained preparation, µL | Limit of detection, copies/sample |
|---|---|---|---|
| Scrapes of epithelial cells in 500 µL of transport medium[11]; urine (1.0 mL) | **PREP-NA** | 50 | 50 |
| | **PREP-NA PLUS** | 300 | 300 |
| | **PREP-GS** | 100 | 100 |
| | **PREP-GS PLUS** | 300 | 300 |
| | **PREP-RAPID** | 500 | 500 |
| | **PREP-MB RAPID**[12] | 100 | 100 |
| | **PREP-MB-RAPID II** | 100 | 100 |
| | **PREP-OPTIMA** | 400 | 400 |

[11] "DNA-Technology" produced transport medium STOR-F was used

[12] Only for scrapes of epithelial cells

### c. Diagnostic characteristics

| Biomaterial | Analyte | Diagnostic sensitivity | Diagnostic specificity |
|---|---|---|---|
| Scrapes of epithelial cells from the urogenital tract | HSV1 | 100% (95% CI: 86.28% – 100%) | 100% (95% CI: 86.28% – 100%) |
| | HSV2 | 100% (95% CI: 86.28% – 100%) | 100% (95% CI: 86.28% – 100%) |
| | CMV | 100% (95% CI: 86.28% – 100%) | 100% (95% CI: 86.28% – 100%) |
| Urine | HSV1 | 100% (95% CI: 86.28% – 100%) | 100% (95% CI: 86.28% – 100%) |
| | HSV2 | 100% (95% CI: 86.28% – 100%) | 100% (95% CI: 86.28% – 100%) |
| | CMV | 100% (95% CI: 86.28% – 100%) | 100% (95% CI: 86.28% – 100%) |

### d. Precision and repeatability

Precision amounts to 100%.
Repeatability amounts to 100%.

## 12. TROUBLESHOOTING

Table 9. Troubleshooting

| | Result | Possible cause | Solution |
|---|---|---|---|
| C+ | - | Operation error<br>PCR inhibition<br>Violation of storage and handlingrequirements | Repeat whole test<br>Dispose current batch |
| C- | + | Contamination | Dispose current batch<br>Perform decontamination procedures |
| IC | Invalid | PCR inhibition | Repeat whole test<br>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 controlprocedures 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 with quality issues of **HSV1/HSV2/CMV Multiplex REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

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## 14. KEY TO SYMBOLS

| | | | |
|---|---|---|---|
| RUO | Research use only |  | Date of manufacture |
|  | Temperature limit |  | Consult instructions for use |
|  | Contains sufficient for <n> tests | REF | Catalogue number |
|  | Use-by date |  | Manufacturer |
| LOT | Batch code |  | Keep away from sunlight |
| VER | Version |  | Caution |
|  | Non-sterile | | |

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