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IVD

For professional use only

HPV 16,18 Multiplex REAL-TIME PCR Detection Kit

INSTRUCTION FOR USE

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REF R1-P320-S3/9EU
R1-P320-23/9EU

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1. INTENDED USE

The **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit** is intended for detection and typing of two most oncogenic and persistent high-risk human papilloma virus types (HPV 16, HPV 18) in human biological samples (epithelial cell scrapes from urethra, cervical canal, cervix) by method of multiplex Real-Time PCR.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit.**

The **HPV 16,18 Multiplex 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 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. The polymerase chain reaction starts only when paraffin is melted. It excludes non-specific annealing of primers to targets DNA in the initial heating of the tube.

The PCR-mix contains target-specific probes, each of them bearing reporter fluorescent dyes 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 HPV product amplification includes fluorescent dyes Fam and Cy5. 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	Hex	Rox	Cy5	Cy5.5
HPV 18	IC	-	HPV 16	-

The automatic analysis is available on “DNA-Technology” made instruments DTlite or DTprime REAL-TIME Thermal Cyclers for **HPV 16,18 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.

3. CONTENT

The **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Tables 2-3.

Table 2. The **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), strips for R1-P320-S3/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white waxy fraction	1920 µL (20 µL in each tube)	12 8-tube strips
Taq-polymerase solution	Colorless transparent liquid	500 µL	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	2 tubes
Positive control*	Colorless transparent liquid	150 µL	1 tube
Strip's caps	12 8-caps		

* - marking as C+ is allowed

Table 3. The **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit** content, package S (standard), tubes for R1-P320-23/9EU

Reagent	Description	Total volume	Amount
Paraffin sealed PCR-mix	Colorless transparent liquid under white waxy fraction	1920 µL (20 µL in each tube)	96 tubes
Taq-polymerase solution	Colorless transparent liquid	500 µL	2 tubes
Mineral oil	Colorless transparent viscous oily liquid	1.0 mL	2 tubes
Positive control*	Colorless transparent liquid	150 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 including no more than 94 experimental samples, negative control and positive control samples.

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.1. Specimen collection

- Sterile single use swabs, cytobrushes, cotton swabs e.t.c for sampling of biomaterial;
- Sterile tubes containing transport medium: "DNA-Technology" made **PREP-RAPID** (REF P-001/1EU,not applicable to male urethral smears) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or **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 16000);
- Solid-state thermostat (temperature range 25-98 °C);
- 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-RAPID** (REF P-001/1EU) (not applicable to male urethral swabs), **PREP-NA** (REF P-002/1EU), **PREP-GS** (REF P-003/1EU) 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;
- Vortex mixer;
- Vortex rotor for strips (in case of using package in strips REF R1-P320-S3/9EU);
- Tube rack for 1.5 mL tubes;
- PCR tube rack for 0.2 mL tubes or strips;
- 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);
- 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 **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit** must be stored at temperatures from 2 °C to 8 °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 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.

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;
- 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 **HPV 16,18 Multiplex 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 **HPV 16,18 Multiplex 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 **HPV 16,18 Multiplex 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

Epithelial cell scrapes from urethra, cervical canal, cervix can be used for analysis.

Sampling, sample processing procedures and storage are carried out in accordance with the instructions to the DNA extraction kit from biological material. **PREP-RAPID** (not applicable to male urethral swabs)**, PREP-NA, PREP-GS** and **PREP-MB RAPID** extraction kits are recommended.

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

To interpret results successfully and robustly, a high quality of sample and appropriate conditions of storage, transport, and handling are required.

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.

Professional prescription is required to localize the place of sampling. The decision must be based on a patient's complaints and clinical signs, and made by the physician in charge.

Women should not carry out genitals toilet and vaginal douching the day before research. To obtain an objective result, it is necessary that the material contains the largest count of epithelial cells and the minimum amount of mucus and blood impurities. Incorrect intake of biological material can lead to uncertain results and, therefore, to re-sample of biomaterial.

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.

Genitourinary scrapes

The features of the posterior vaginal vault sampling

The material should be taken before the physical inspection. The speculum before manipulation can be moistened with hot water; the use of antiseptics for speculum treatment is contraindicated. Scraping is taken from the posterior vaginal vault. In case of virginal women, scraping is taking from the vestibular mucous membrane and in some cases from the posterior vaginal vault through hymenal rings.

The features of the cervical sampling

Before sampling procedure, it is necessary to remove the mucus with a cotton tampon and, then, treat the cervix with a sterile physiological solution. The sampling swab is inserted into the cervical canal to a depth of 0.5 – 1.5 cm. Removing the swab, contact of the walls of the vagina should be excluded.

The features of cervix uteri sampling

The sample must be taken prior to physical inspection.

Before taking the material, remove the mucus, inflammatory exudate or blood (if any) with a sterile cotton swab.

The exfoliative cellular material and the superficial epithelium should be carefully scraped off from the vaginal portion of the cervix, the area of the transformation zone (CT) and/or the cervical canal if the connection zone of the stratified squamous epithelium and cylindrical epithelium is moved into the cervical canal.

The features of the urethral sampling

Before sampling procedure, the patient is recommended to refrain from urination for 1.5 – 2 hours.

Immediately before sampling procedure, it is necessary to treat the external urethral orifice with a tampon moistened with sterile physiological solution.

In the presence of purulent discharge, the sample must be taken 15-20 minutes after urination. In the absence of discharge, it is necessary to massage the urethra with sampling swab or brush. In case of women, the swab or brush is inserted to a depth of 1.0-1.5 cm, in case of children; the material is taken only from the external urethral orifice.

Genitourinary scrapes sampling (cervical canal, cervix uteri, vagina, urethra)

Procedural limitations - local application of medicines, vaginal ultrasound less than 24 hours before the procedure. Women must not perform hygiene procedures or syringing prior the sampling procedure.

Sampling procedure is carried out using special sterile disposable instruments – urogenital swabs, cytobrushes or tampons, depending on the source of clinical material in accordance with established procedures. The sampling using a device for self-sampling is carried out in accordance with the instructions for use of the device.

ATTENTION! In case of pregnancy the use of cytobrushes is contraindicated.

The taking of the smears is carried out:

- in plastic 1.5 mL tubes with 300-500 µL of a sterile physiological solution;
- in tubes with transport medium intended by the manufacturer for transportation and storage of samples for PCR.

Order of taking:

1. Open the tube.
2. Scrape epithelial cells from the corresponding biotope (i.e. vagina, urethra, cervical canal) with a sterile swab.
3. Put the swab into the tube with transport medium and rinse it thoroughly. Avoid spraying of solution.
4. Remove swab from solution, press it to the wall of tube and squeeze the rest of the liquid. Throw out the swab.
5. Close the tube tightly and mark it.

Samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Transportation and storage of samples

Samples taken in sterile physiological solution are stored at temperatures from 2 °C to 8 °C for no more than 24 h. 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.

Samples are stored according to the instruction for the transport medium used intended for subsequent sample analysis by PCR.

Sample preparation

It is necessary to perform pretreatment before DNA extraction by the in **PREP-RAPID** (not applicable to male urethral swabs)**, PREP-NA, PREP-GS** and **PREP-MP RAPID** kits. It is allowed to use any kits of reagents registered as a medical device and recommended by manufacturers for the extraction of DNA from the corresponding types of biomaterial.

ATTENTION! No additional pretreatment is required in case of taking scrapes into tubes with the "PREP-RAPID" reagent.

Genitourinary scrapes

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

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

The resulting material is ready for DNA extraction.

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

8. PROCEDURE

DNA extracting from biological material

DNA extraction is carried out in accordance with the instruction to the extraction kit. DNA extraction kits for subsequent usage of DNA in PCR and intended for corresponding types of biomaterial are recommended.

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

Preparing PCR for package S

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

ATTENTION! When using package S (R1-P320-S3/9EU), strips, 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 Mark the required number of tubes with paraffin sealed PCR-mix 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 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds to collect the drops.

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

8.4 Add one drop (~20 µL) of mineral oil into each tube.

8.5 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 strip to prevent contamination. Close the tubes/strips tightly. Use filter tips.

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

8.7 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.

8.8 Spin tubes/strips for 3-5 seconds.

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

8.10 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 8.9) and run PCR. See Table 4.

Table 4. 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[1]			Holding		Holding
[1] – holding at 25°C is allowed						

9. CONTROLS

The **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit** contains positive control sample. Positive control is a cloned part of the HPV 16, HPV 18 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.

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 sample in volumes indicated in supplied instructions.

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.

[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.

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.

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

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

The resulting graph will display the dependence of fluorescence intensity on the cycle number for each tube. Type of the sample, name of the test, value of the threshold cycle (Cp) and test result (qualitative) will be displayed in the right module of the window.

The results of the analysis can be used to generate and print a report.

Interpretation of the PCR results should be performed according to the Table 5.

Table 5. Interpretation of the PCR results

Detection channel			Result	Result interpretation
Fam	**Hex**	**Cy5**		
Analyzed samples				
Cp is specified	Is not considered	Cp is specified	+	DNA HPV16 and HPV18 is detected
Cp is not specified	Is not considered	Cp is specified	+	DNA HPV16 is detected
Cp is specified	Is not considered	Cp is not specified	+	DNA HPV18 is detected
Cp is not specified	Cp is specified	Cp is not specified	-	DNA HPV16 and HPV18 is not detected
Cp is not specified	Cp is not specified	Cp is not specified	Invalid	Invalid result*
Positive control sample				
Cp is specified	Is not considered	Cp is specified	+	Positive result. The results are valid
Negative control sample				
Cp is not specified	Cp is specified	Cp is not specified	-	Negative result. The results are valid

* In this case amplification, or DNA extraction, or collecting of clinical material are required to be repeated (performed sequentially).

In case of results for negative control sample different from those in Table 5, the results of all series are considered invalid. In this case decontamination procedures are required.

In case of results for positive control sample different from those in Table 5, it is required to repeat amplification for all series.

11. SPECIFICATIONS

a. The analytical specificity of the **HPV 16,18 Multiplex 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 HPV DNA are to be registered positive for specific product (a fragment of the HPV genome). The samples free of HPV DNA are to be registered negative for specific product and positive for internal control.

There are not non-specific positive results of amplification of DNA sample in the presence of *Ureaplasma urealyticum*, *Ureaplasma parvum, Gardnerella vaginalis*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Chlamydia trachomatis*, *Candida albicans*, *Streptococcus sp*., *Staphylococcus sp*., *Lactobacillus spp*., EBV, HHV6, HHV8, HSV1, HSV2, VZV.

b. Analytical sensitivity

Detection threshold is 5 copies of DNA on amplification tube. Detection threshold was established by a series of dilution of laboratory control sample (LCS).

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

12. TROUBLESHOOTING

Table 6. Troubleshooting

	Result	Possible cause	Solution
C+	-	Operation error PCR inhibition Violation of storage and handlingrequirements	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 issueswith 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 IVDD products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service by quality issues of **HPV 16,18 Multiplex REAL-TIME PCR Detection Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

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

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

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
CONTROL +	Positive control	VER	Version
	Caution		

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