DNA-TECHNOLOGY

769-2 2024-09-03

CE IVD

For professional use only

HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit

INSTRUCTION FOR USE

EC REP

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"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

Customer service department

E-mail: hotline@dna-technology.ru

REF R1-P325-S3/9EU
R1-P325-23/9EU

VER 769-2.2024.09.03

TABLE OF CONTENTS

1. INTENDED USE

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is intended for research and diagnostic applications. The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is an *in vitro* Nucleic Acid Test (NAT) – pathogen-detection-based product. The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is designed to detect HPV nucleic acids in human biological samples with an aid of Polymerase Chain Reaction (PCR) method. Samples are human biological materials: epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina.

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is designed for qualitative DNA detection of fourteen types of human papillomaviruses with high carcinogenic risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 types) associated with cervical cancer, and differential detection of HPV 16, 18 and 45 types in human biological material.
Indications for the use: screening for precancer and cervical cancer, symptoms of HPV infection.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit.**

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR 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

Method: polymerase chain reaction (PCR) with detecting of the results in real time; qualitative multiplex 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. Hot-start is provided by reaction mixture preparation consisting of two layers separated by paraffin layer. 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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is based on fluorescent modification of the PCR method. The PCR-mix contains target-specific probes 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.

Amplification mixture includes a system for amplification of a fragment of human genomic DNA (sample intake control, SIC), which allows to evaluate the presence of DNA in the amplification tube and control all stages of the analysis.

DNA probes used to detect specific DNA amplification products include the fluorescent tags Fam, Rox, Cy5, Cy5.5. The DNA probe used to detect the amplification product of the sample intake control includes the fluorescent dye Hex.

The application of several fluorescent dyes makes it possible to register the results of different amplification reactions taking place simultaneously in one tube. Table 1 shows the detection channels of amplification products.

Table 1. Detection channels of amplification products

Fam	Hex	Rox	Cy5	Cy5.5
16	SIC	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68	18	45

The automatic analysis is available on "DNA-Technology" made instruments: DTlite[1] or DTprime[2] REAL-TIME Thermal Cyclers for **HPV SCREEN HR14(16-18-45) REAL-TIME PCR 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 SCREEN HR14(16-18-45) REAL-TIME PCR Kit** contains PCR-mix, Taq-polymerase solution, mineral oil and positive control sample. The detailed description of content is represented in Table 2-3.

Table 2. The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** content, package S (standard), strips, for R1-P325-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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** content, package S (standard), tubes R1-P325-23/9EU

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

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

The **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is intended for single use and designed for 96 tests (no more than 94 defined samples, one positive control and one negative control).

[1] - supported by 5S1, 5S2 instruments.
[2] - supported by 5M1, 5M3, 5M6 instruments.
If using detecting thermal cyclers with 4 detection channels, papilloma type 45 is not detected.

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 **STOR-M** (REF P-910-1/1EU) or **STOR-F** (REF P-901-1/1EU, P-901-N/1EU, P-901-R/1EU) or equivalent or physiological saline solution or sterile PBS 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 50-65 °C);
- Tube rack for 1.5 mL tubes;
- 1.5 mL tubes;
- Single channel pipettes (dispensers covering 1.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL, 20 µL, 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-A/8EU, REF P-116-N/4EU 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;
- PCR tube rack for 0.2 mL tubes;
- PCR tube rack for strips of eight 0.2 mL tubes;
- Single channel pipettes (dispensers covering 0.5-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 10 µL, 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 SCREEN HR14(16-18-45) REAL-TIME PCR Kit** must be stored 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 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.

It is allowed to transport the kit in thermobox 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 under undue regime should not be used.

An expired the **HPV SCREEN HR14(16-18-45) REAL-TIME PCR 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 SCREEN HR14(16-18-45) REAL-TIME PCR 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 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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR Kit** is designed to detect DNA extracted from the epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina.

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

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.

The maximum concentrations of interfering substances at which no PCR inhibition was observed are shown in the table below:

Type of biomaterial	**Interfering substance**	**Studied concentration in the sample**
Endogenous substances		
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Hemoglobin*	0.35 mg/mL
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Mucus (mucin)	20 %
Exogenous substances		
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Isopropyl alcohol*	10%
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Methyl acetate*	10%
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Chlorhexidine bigluconate	10%
epithelial smears/scrapes from the mucous membrane of the cervical channel and the vagina	Miramistin®	10%

*** -** interfering substance may be present in DNA preparation due to its incomplete removement in the course 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.

Sample collection

Epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina

Sample taking is made with special sterile single-use tools – probes, cytobrushes, swabs depending on the source of biological material according to established procedure.

ATTENTION! In case of pregnancy the use of cytobrushes for genitourinary smears sampling is contraindicated.

It is allowed to use swabs to take the material on your own.

Procedural limitations - local application of medicines, vaginal ultrasound less than 24 hours before the procedure.

The taking of the swabs is carried out 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 Take biological material with a sterile swab.

3 Put the swab into the tube with transport medium and rinse it thoroughly for 10-15 seconds. 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.

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.

Before sampling procedure, it is necessary to remove the mucus with a cotton tampon.

The features of the vaginal sampling using a device for self-sampling

The sampling is carried out in accordance with the instructions for use of the device.

Material is taken into the transport & fixation medium for liquid cytology in accordance with the Instruction for the transport & fixation medium for liquid cytology.

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

Transportation and storage of the samples

Transport and storage conditions for epithelial smears/scrapes from the mucous membrane of the cervical canal and the vagina are determined by the instructions for the transport media used for sample transport and storage or the instructions for the recommended DNA extraction kits.

Sample preparation

Epithelial smears/samples from the mucous membrane of the cervical canal and the vagina, collected in transport media for PCR studies.

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.

Vaginal material taken using a self-sampling device

1 Add 500 µL of physiological saline solution to the tube (or other container specified in the instructions for the self-sampling device ("tube") that holds the tip of the device.

2 Shake the tube for 15 seconds.

3 Remove the tip of the device from the tube and discard it.

4 Transfer to a clean 1.5 mL tube.

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

6 Remove the supernatant, leaving approximately 100 µL in the tube (precipitate+liquid fraction).

The resulting material is ready for DNA extraction.

Scrapes taken in transport & fixation medium for liquid cytology

When fixing in some alcoholic transport media for liquid cytology, for example, in the preservative liquid BD SurePath (Becton Dickinson, USA), cross-linking of nucleic acids with proteins occurs, so pre-treatment of samples in order to release DNA from protein-associated complexes and cell lysis is necessary.

Scrapes taken in the transport & fixation medium for liquid cytology BD SurePath (Becton Dickinson, USA) must be pretreated using the **PREP-PK** reagent kit ("DNA-Technology", LLC) in accordance with the instructions to the **PREP-PK** reagent kit.

Transport & fixation media PreservCyt®ThinPrep (Hologic Inc, USA) or CellPrep (Biodyne, Korea) do not require sample pretreatment using the **PREP-PK** reagent kit.

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. 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! Independently of DNA extraction kit used, a negative control sample 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.

Assay procedure

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

ATTENTION! When using package S (R1-P325-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 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.

8.2 Vortex the Taq-polymerase solution for 3-5 seconds, then spin for 1-3 seconds.

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. Close tubes/strips.

8.5 Vortex the tubes with samples, “C-” and “C+” for 3-5 seconds, then 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 Extraction Kit**. The DNA samples must stand in a magnetic rack while adding DNA. 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.

ATTENTION! Open 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 µL of DNA sample into corresponding tubes. Do not add DNA into the "C-" and "C+" tubes. Avoid paraffin layer break.

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

8.8 Add 5.0 µL of positive control (C+) into "C+" tube. Avoid paraffin layer break.

8.9 Spin tubes/strips for 3-5 seconds.

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

8.11 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 8.10) 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 SCREEN HR14(16-18-45) REAL-TIME PCR Kit** contains positive control sample. Positive control is a cloned part of the HPV genome. It is produced with genetic engineering techniques and characterized by automatic DNA sequencing. The PCR-mix contains sample intake control (SIC). Sample intake control (SIC) estimates the amount of human DNA in the tube. 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 from an extraction kit can be used as a negative control sample in volumes indicated in supplied instructions.

[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.

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

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

10. DATA ANALYSIS

Registration of the PCR results is held in automatic mode. Analysis will be performed by Real-Time PCR application. The resulting graph will display the dependence of fluorescence intensity on the cycle number for each tube. Operator can create, save and print a report.

When the PCR is complete, the program displays a "+" or "-" in the table in the "Result" column. In this case a conclusion on the results of the study is given.

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

Table 5. Interpretation of the PCR results

Detection channel					**Interpretation**
Fam	**Hex**	**Rox**	**Cy5**	**Cy5.5**	
+	Is not considered	+	-	-	DNA HPV16 is detected
-	Is not considered	+	+	-	DNA HPV18 is detected
-	Is not considered	+	-	+	DNA HPV45 is detected
-	Is not considered	+	-	-	DNA HPV 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68 is detected (no differentiation)
-	+	-	-	-	DNA HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 is not detected
-	-	-	-	-	Invalid result*
Positive control sample					
+	+	+	+	+	The results are valid
Negative control sample					
-	-	-	-	-	The results are valid

* - Invalid results can be due to the presence of inhibitors in the DNA preparation obtained from biological material; incorrect performance of the analysis protocol; failure to follow the amplification temperature regime, etc. In this case, it is necessary to repeat PCR with the existing DNA preparation, or repeat DNA extraction and PCR, or repeat collection of biological material (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 this case special measures to detect and eliminate possible contamination 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 SCREEN HR14(16-18-45) REAL-TIME PCR 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.

In samples of human biological material containing human papillomavirus type 16 DNA, the detection thermocycler software records positive amplification results for the specific product on the Fam and Rox channels during amplification.

In samples of human biological material containing human papillomavirus type 18 DNA, detection thermocycler software records positive amplification results for the specific product on the Cy5 and Rox channels during amplification.

In samples of human biological material containing human papillomavirus type 45 DNA, detection thermocycler software detects positive amplification results for the specific product on the Cy5.5 and Rox channels during amplification.

In samples of human biological material that contain DNA from one or more human papillomavirus (HPV) types at high carcinogenic risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), the detection thermocycler software records positive amplification results for the specific product on the Rox channel during amplification.

In samples of biological material that do not contain DNA of 14 types of highly carcinogenic human papillomaviruses (HPV) (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68), the detection thermocycler software detects negative amplification results for the specific product on the Fam, Rox, Cy5, and Cy5.5 detection channels and positive amplification results for SIC on the Hex detection channel.

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**

Analytical sensitivity is 5 DNA copies of each type of human papillomavirus per amplification tube, which corresponds to 10^3 DNA copies of each type of human papillomavirus per 1.0 mL.

c. Diagnostic characteristics

Number of samples (n) - 436

Analyte detected	Diagnostic sensitivity (95% CI)	Diagnostic specificity (95% CI)
HPV16	100.00% (96.38%-100.00%)	100.00% (98.91%-100.00%)
HPV18	100.00% (83.16%-100.00%)	100.00% (99.12%-100.00%)
HPV45	100.00% (69.15%-100.00%)	100.00% (99.14%-100.00%)
HPV 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68 (no differentiation) (excluding types 16, 18 and 45)	100.00% (98.54%-100.00%)	100.00% (98.04%-100.00%)

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-A/8EU, REF P-116-N/4EU extraction kits.

12. TROUBLESHOOTING

Table 6. 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
SIC	-	PCR inhibition Insufficient amount of DNA	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

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 **HPV SCREEN HR14(16-18-45) REAL-TIME PCR 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

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

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
EC REP	Authorized representative in the European Community		Caution

REF	R1-P325-S3/9EU R1-P325-23/9EU	VER	769-2.2024.09.03