RUO

For Professional Use Only

AmpliSens® *HPV* HCR screen-FEP

PCR kit

Instruction Manual

AmpliSens®

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1. INTENDED USE

AmpliSens® *HPV* HCR screen-FEP PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of high carcinogenic risk (HCR) *human papillomaviruses* (*HPV*) types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67 DNA in the biological material (cervical and urethral scrapes) by using end-point hybridization-fluorescence detection.

The PCR kit is intended for qualitative detection (without genotyping) of DNA of *HPV* HCR types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67. When using PCR kit variant screen-FEP 2x the separate detection of DNA of *HPV* type 16 is performed. These types have a high transforming activity and are the cause of more than 92 % of high-grade cervical dysplasia and cervical cancer. The endogenous internal control (a fragment of a human β-globin gene) is used in the PCR kit.

For research use only. Not for diagnostic procedures.

2. PRINCIPLE OF PCR DETECTION

HPV types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special primers. In Fluorescent End-Point PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes that bind specifically to the amplified product during thermocycling. A multichannel rotor-type fluorometer is specially designed to detect fluorescent emission from the fluorophores in the reaction mixture after the PCR. It allows the detection of the accumulating product without re-opening the reaction tubes after the PCR run.

The test is based on simultaneous amplifying (multiplex PCR) and end-point detection of DNA fragments of *HPV* and a fragment of β-globin gene which is used as an endogenous internal control. Test identifying eleven types of *HPV* HCR is running either in a single tube or two tubes depending on the variant of PCR kit.

The DNA-target selected as an endogenous internal control is the fragment of human genome and must be present in a sample (cervical scrape) in sufficient quantity equivalent to that of cells in the sample (10^3-10^5 genomes). Therefore, the use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and amplification) but also to assess the adequacy of sampling and storage of biological material. In case of incorrect sampling of epithelial scrape (insufficient amount of epithelial cells), amplification signal of β-globin gene will be too low.

AmpliSens® *HPV* HCR screen-FEP PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using

chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

Detection of clinically significant virus quantity by using of AmpliSens® *HPV* HCR screen-FEP PCR kit

According to epidemiologic studies most routine screening examinations for dysplastic changes of cervix, vagina, and vulva as well as risk of their development require detection of *clinically valuable* quantity of high carcinogenic risk human papillomavirus. Believed, that detection of virus in quantity not exceeding certain threshold value is clinically insignificant because 100% of such cases associate with spontaneous recovery. On the contrary, high virus load suggests about dysplasia or risk of its development. However, in case of monitoring of treatment, diagnosis of even low virus load can marker an early relapse. Currently, level of clinically significant virus quantity estimates at 10^5 GE of HCR *HPV* per cervical scrape when standardized obtaining of biological material is provided. Investigations done on model samples have showed that only clinically significant virus quantity is detected if following steps are applied:

- collection of cervical scrape by standard procedure (placed in 0.5 ml of transport medium)
- DNA extraction (DNA-sorb-AM was used);
- 100x dilution of obtained DNA in TE-buffer;
- PCR-test.

Trials of this approach on specimens collected from both healthy patients and patients suffering from severe dysplasia and cervical cancer demonstrated increase of specificity of dysplasia detection by 22.9% (from 61.7% without dilution to 84.6% if dilution was applied) while high level of severe dysplasia and cervical cancer diagnosis was maintained (98.9%). Note that level of clinically significant virus quantity wasn't validated for men.

Therefore, **AmpliSens® *HPV* HCR screen-FEP** PCR kit allows two formats of HCR *HPV* detection:

- presence of *HPV* HCR (sample to be tested after DNA extraction)
- clinically significant quantity of *HPV* HCR (sample to be tested after DNA extraction and dilution in TE-buffer). Note that standardized obtaining of biological material is necessary.

3. CONTENT

AmpliSens® *HPV* HCR screen-FEP PCR kit is produced in 2 forms:

AmpliSens® *HPV* HCR screen-FEP PCR kit variant screen-FEP 2x, **REF** V31-FEP-CE.

AmpliSens® *HPV* HCR screen-FEP PCR kit variant screen-FEP 3x, **REF** V31-3x-FEP-CE.

AmpliSens® *HPV* HCR screen-FEP PCR kit variant screen-FEP 2x includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP *HPV*-1 (per 30 reactions)	clear liquid from colorless to light lilac colour	0.21	4 blue cap tubes
PCR-mix-1-FEP *HPV*-2 (per 30 reactions)	clear liquid from colorless to light lilac colour	0.21	4 green cap tubes
PCR-buffer-Flu (per 56 reactions)	colorless clear liquid	0.42	4 tubes
Polymerase (TaqF) (per 56 reactions)	colorless clear liquid	0.028	4 tubes
PCR-mix-Background *HPV*	colorless clear liquid	0.8	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 vial
Positive Control DNA *HPV* types 16, 31, 33 and human DNA (C+$_{HPV\ 16,31,33}$)	colorless clear liquid	0.2	2 tubes
TE-buffer	colorless clear liquid	5.0	5 tubes
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (**DNA-sorb-AM**, **REF** K1-12-100-CE, **DNA-sorb-B**, **REF** K1-2-100-CE, **DNA-sorb-C**, **REF** K1-6-50-CE, **AmpliSens® DNA-sorb-D** **REF** K8-2331-100-CE protocol).

AmpliSens® *HPV* HCR screen-FEP PCR kit variant screen-FEP 2x is intended for 120 reactions, including controls.

AmpliSens® *HPV* HCR screen-FEP PCR kit variant screen-FEP 3x includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FEP *HPV* 3x (per 30 reactions)	clear liquid from colorless to light lilac colour	0.21	4 tubes
PCR-buffer-Flu (per 56 reactions)	colorless clear liquid	0.42	2 tubes
Polymerase (TaqF) (per 56 reactions)	colorless clear liquid	0.028	2 tubes
PCR-mix-Background *HPV*	colorless clear liquid	0.8	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 vial
Positive Control DNA *HPV* types 16, 31, 33 and human DNA (C+$_{HPV\ 16,31,33}$)	colorless clear liquid	0.2	1 tube
TE-buffer	colorless clear liquid	5.0	5 tubes
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

* must be used in the extraction procedure as Negative Control of Extraction (**DNA-sorb-AM**, **REF** K1-12-100-CE, **DNA-sorb-B**, **REF** K1-2-100-CE, **DNA-sorb-C**, **REF** K1-6-50-CE, **AmpliSens® DNA-sorb-D** **REF** K8-2331-100-CE protocol).

AmpliSens® *HPV* HCR screen-FEP PCR kit variant screen-FEP 3x is intended for 120 reactions, including controls.

4. ADDITIONAL REQUIRMENTS

- DNA extraction kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol filters up to 200 µl.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with a rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cycler (Corbett Research, Australia), MaxyGene (Axygene, USA)).
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene tubes for PCR with 0.2 (0.5) ml capacity (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer for the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all positive material (specimens, controls) away from all other reagents and add it to the reaction mix in a distantly separated facility. Store and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge them briefly.
- Use disposable protective gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.

- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with the local regulations.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section “Content”).
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment, and reagents to the area where the previous step was performed.

Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

Obtaining samples of biological materials for the PCR-analysis, transportation and storage are described in the manufacturer’s handbook [1]. It is recommended that this handbook is read before starting work.

AmpliSens® *HPV* HCR screen-FEP PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from the biological material (cervical or urethral scrapes).

Female: samples of epithelial cells should be obtained as for cytological examination.

Method 1. The sampling kit with one/two cervical cytobrushes and 2-ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** REF 953-CE are used.

Place the cervical epithelial scrape (endocervix) taken with the first cervical cytobrush

and/or the superficial cervical scrape (ectocervix) taken with the second cervical cytobrush to the tube with transport medium. Break the lower part of the cytobrush and leave it in the tube with transport medium.

Method 2. The Digene cervical sampler (USA), which contains cervical cytobrush and a tube with 1.0 ml of Digene transport medium, is used.

Place the cervical epithelial scrape (endocervix) obtained with cytobrush into the tube with Digene transport medium.

Method 3. The sampling kit, which contains the combined gynecological probe for simultaneously taking epithelium from endocervix and ectocervix and 5-ml tube with 2.0 ml of **Transport Medium with Mucolytic Agent** **REF** 952-CE, is used.

Place the endocervix and ectocervix into the tube with transport medium. Break the lower part of the probe and leave it in the tube with transport medium.

Method 4. The sampling kit, which contains the combined gynecological probe for simultaneously taking epithelial samples from endocervix and ectocervix and a liquid-based cytology vial with CytoScreen (Italy) or PreservCyt (USA) transport medium, is used.

Place the endocervix and ectocervix into the tube with transport medium. Break the lower part of the probe and leave it in the vial with transport medium

Male: Obtain urethral epithelial scrape by universal probe, place it into the 2.0 ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** **REF** 953-CE.

Storage conditions:

- at the temperature from 18 to 25 °C – no more than 5 days;
- at the temperature from 2 to 8 °C – no more than 20 days;
- at the temperature from minus 24 to minus 16 °C – for 1 year. Only one freeze-thawing cycle is allowed;
- in the transport medium for liquid-based cytology at room temperature – for 1 year.

7. WORKING CONDITIONS

AmpliSens® *HPV* HCR screen-FEP PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA extraction

It's recommended to use the following nucleic acid extraction kits:

- **DNA-sorb-AM,** REF K1-12-100-CE (for the biological material obtained by the 1st, 2nd and 3rd methods);
- **DNA-sorb-B**, REF K1-2-100-CE (for the biological material obtained by the 1st, 2nd and 3rd methods);
- **DNA-sorb-C**, REF K1-6-50-CE (for biopsy materials);
- **AmpliSens® DNA-sorb-D** REF K8-2331-100-CE (for samples for liquid-based cytology).

In the extraction procedure it is necessary to carry out the control reaction as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative control of Extraction).

Extract the DNA according to the manufacturer's protocol.

<u>Variant screen-FEP 2x</u>

8.2. Preparing of the PCR

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

This variant applies two mixes of primers and probes:

- **PCR-mix-1-FEP *HPV*-1** is intended for amplification and detection of DNA of *HPV* genotypes 16, 31, 35, 39 and 59. Genotypes 31, 35, 39 and 59 are detected in the channel for the FAM fluorophore (channel 1 of ALA-1/4 fluorescence detector). Genotype 16 is detected separately in the channel for the JOE fluorophore (channel 2 of ALA-1/4 fluorescence detector). This mix doesn't contain Internal Control.
- **PCR-mix-1-FEP *HPV*-2** is intended for amplification and detection of DNA of *HPV* genotypes 18, 33, 45, 52, 58 and 67 as well as the fragment of human β-globin gene (endogenous internal control). Genotypes 18, 33, 45, 52, 58 and 67 are detected in the channel for the FAM fluorophore (channel 1 of ALA-1/4 fluorescence detector). Endogenous internal control is detected separately in the channel for the JOE fluorophore (channel 2 of ALA-1/4 fluorescence detector).

  DNA amplification should be performed by applying both PCR-mix-1-FEP *HPV*-1 and PCR-mix-1-FEP *HPV*-2. The result is considered positive if positive signal is detected at least for one of the PCR-mixes-1-FEP.

8.2.1 Preparing of tubes for PCR.

1. Prepare **PCR-buffer-Flu** and **polymerase (TaqF)** mixture. To do this, transfer the content of one tube with **polymerase (TaqF) (28 µl)** to the tube with **PCR-buffer-Flu**

(420 µl) and carefully vortex. Avoid foaming while mixing. Indicate the time of the mixture preparation on the tube.

The prepared mixture is intended for **56** reactions. The mixture should be stored at 2-8 °C for 3 months and used as needed.

2. Prepare the reaction mixtures (see table 1). When calculating take into account that every run should include two controls for each mix (**negative and positive controls**). Moreover, it is necessary to add reagents for one extra reaction.
3. Each PCR reaction should include:
   - **7 µl** of **PCR-mix-1-FEP *HPV*-1 or PCR-mix-1-FEP *HPV*-2;**
   - **8 µl of PCR-buffer-Flu and polymerase (TaqF) mix.**

Table 1

Reaction mixes preparation scheme

Number of samples	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
PCR-mix-1-FEP *HPV*-1/ *HPV*-2, µl	28	35	42	49	56	63	70	77	84	91	98	105	112
PCR-buffer-Flu and polymerase (TaqF) mixture, µl	32	40	48	56	64	72	80	88	96	104	112	120	128
Number of samples	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
PCR-mix-1-FEP *HPV*-1/ *HPV*-2, µl	119	126	133	140	147	154	161	168	175	182	189	196	203
PCR-buffer-Flu and polymerase (TaqF) mixture, µl	136	144	152	160	168	176	184	192	200	208	216	224	232

Calculation for PCR-mix-1-FEP *HPV*-1 is the same as for PCR-mix-1-FEP *HPV*-2.

4. Insert in a tube rack two PCR-tubes for each test sample, two tubes for positive control, and two tubes for negative control. Transfer 15 µl of reaction mixture, containing PCR-mix-1-FEP *HPV*-1 per half of the tubes (per each tube). To the remaining half of the tubes add 15 µl of reaction mixture, containing PCR-mix-1-FEP *HPV*-2 (per each tube).
5. Add above 1 drop of mineral oil for PCR[1].
6. Prepare **2 background samples per each PCR-mix-1**.
   - **If one of the recommended DNA extraction kits is used,** into two PCR tubes transfer 7 µl of PCR-mix-1-FEP *HPV*-1 and 18 µl of PCR-mix-Background *HPV* (per

[1] The addition of mineral oil for PCR is not required when using the thermocyclers with constant-temperature lid.

each). Add above 1 drop of mineral oil for PCR[1].

— **If different method is applied for DNA extraction,** into two PCR tubes transfer 7 µl of PCR-mix-1-FEP *HPV*-1, 8 µl of PCR-buffer-Flu, and 10 µl of Negative Control of Extraction (C-) (per each). Add above 1 drop of mineral oil for PCR [1].

Prepare background samples for PCR-mix-1-FEP *HPV*-2 similarly.

Background samples that have been amplified once can be stored at 2-20 ºC for up to 1 month and can be used for further runs. Multiple use of Background samples is permitted in case of the use of the same lot of PCR kit, same extraction kit and the same tube type.

7. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage into prepared pairs of tubes.
8. Carry out the control amplification reactions:

NCA — Add **10 µl** of **TE-buffer** to the pair of tubes labeled NCA (Negative Control of Amplification).

C+ — Add **10 µl** of **Positive Control DNA *HPV* types 16, 31, 33 and human DNA (C+$_{HPV\ 16,31,33}$)** to the pair of tubes labeled C+ (Positive Control of Amplification).

C– – Add **10 µl** of the sample extracted from the **Negative Control (C–) reagent** to the pair of tubes labeled C– (Negative Control of Extraction).

8.2.2 Amplification.

1. Run the following program in the thermocycler (see Table 2).
2. When the temperature reaches 95 °C (pause mode), insert tubes into the wells of the thermocycler and press the button to continue.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

Table 2

Programming thermocycler for DNA amplification of *HPV* types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67

	Thermocyclers with active temperature adjustment (by solution in tube):						**Other termocyclers**		
	GeneAmp PCR System 2700			**Gradient Palm Cycler, MaxyGene**					
step	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles
1	**95 °C**	15 min	1	**95 °C**	15 min	1	**95 °C**	15 min	1
2	**95 °C**	10 s	50	**93 °C**	5 s	50	**95 °C**	25 s	50
	59 °C	20 s		**59 °C**	10 s		**59 °C**	25 s	
	72 °C	10 s		**72 °C**	5 s		**72 °C**	25 s	
3	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
	95 °C	20 s	1	**95 °C**	20 s	1	**95 °C**	20 s	1
4	**10 °C**	storage		**10 °C**	storage		**10 °C**	storage	

3. Proceed to fluorescence detection after the amplification program is completed.

Variant screen-FEP 3x

8.3. Preparing of the PCR

Total reaction volume is **25 µl**, the volume of DNA sample is **10 µl**.

This variant applies a single mixture of primers and probes:

— Genotypes 31, 35, 39 and 59 are detected in the channel for the ROX fluorophore (channel 3 of ALA-1/4 fluorescence detector). Genotypes 16, 18, 33, 45, 52, 58 and 67 are detected in the channel for the FAM fluorophore (channel 1 of ALA-1/4 fluorescence detector), Internal Control is detected separately in the channel for the JOE fluorophore (channel 2 of ALA-1/4 fluorescence detector).

8.3.1 Preparing tubes for PCR.

1. Prepare the mixture of **PCR-buffer-Flu** and **polymerase (TaqF)**. To do this, transfer the content of one tube with **polymerase (TaqF) (28 µl)** to the tube with **PCR-buffer-Flu (420 µl)** and carefully vortex. Avoid foaming while mixing. Indicate the time of the mix preparation on the tube.

The mixture should be stored at 2-8 °C for 3 months and used as needed.

2. Mix following reagents in a separate tube calculating per <u>one</u> reaction:
   — **7 µl** of **PCR-mix-1-FEP *HPV* 3x;**
   — **8 µl** of **mixture of PCR-buffer-Flu** and **polymerase (TaqF).**

When calculating take into account that every run should include two controls (**negative and positive**). Moreover, it is necessary to add reagents for one extra reaction.

3. Insert in a tube rack one PCR-tube for each test sample, one tube for positive control and one tube for negative control. Transfer **15 µl** of reaction mixture per each tube.
4. Add above 1 drop of mineral oil for PCR[2].
5. Prepare **2 background samples**:
   — **If one of the recommended DNA extraction kits is used,** transfer 7 µl of PCR-mix-1-FEP *HPV* 3x and 18 µl of PCR-mix-Background *HPV* into two PCR tubes (per each tube). Add above 1 drop of mineral oil for PCR[2].
   — **If different method is applied for DNA extraction,** transfer 7 µl of PCR-mix-1-FEP *HPV* 3x, 8 µl of PCR-buffer-Flu, and 10 µl of Negative Control of Extraction (C-) into two PCR tubes (per each tube). Add above 1 drop of mineral oil for PCR[2].

Background samples that have been amplified once can be stored at 2-20 ºC for up to 1 month and can be used for further runs. Multiple use of Background samples is permitted in case of the use of the same lot of PCR kit, same

[2] The addition of mineral oil for PCR is not required when using the thermocyclers with constant-temperature lid.

extraction kit and the same tube type.

6. Add **10 µl** of **DNA samples** obtained at the DNA extraction stage.
7. Carry out control amplification reactions:

NCA — Add **10 µl** of **TE-buffer** to the tube labeled NCA (Negative Control of Amplification).

C+ — Add **10 µl** of **Positive Control DNA *HPV* types 16, 31, 33 and human DNA** to the tube labeled C+ (Positive Control of Amplification).

C– – Add **10 µl** of the sample extracted from the **Negative Control (C–) reagent** to the tube labeled C– (Negative Control of Extraction).

8.3.2 Amplification.

1. Run the following program in the thermocycler (see Table 3).
2. When the temperature reaches 95 °C (pause mode), insert tubes into the wells of the thermocycler and press the button to continue.

It is recommended to sediment drops from walls of tubes by short centrifugation (1–3 s) before placing them in the thermocycler.

Table 3

Programming thermocycler for DNA amplification of *HPV* types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67

	Thermocyclers with active temperature adjustment:						**Other thermocyclers**		
	GeneAmp PCR System 2700			**Gradient Palm Cycler, MaxyGene**					
step	temperature	time	cycles	temperature	time	cycles	temperature	time	cycles
1	**95 °C**	15 min	1	**95 °C**	15 min	1	**95 °C**	15 min	1
2	**95 °C**	10 s	50	**93 °C**	5 s	50	**95 °C**	25 s	50
	59 °C	20 s		**59 °C**	10 s		**59 °C**	25 s	
	72 °C	10 s		**72 °C**	5 s		**72 °C**	25 s	
3	**72 °C**	1 min	1	**72 °C**	1 min	1	**72 °C**	1 min	1
	95 °C	20 s	1	**95 °C**	20 s	1	**95 °C**	20 s	1
4	**10 °C**	storage		**10 °C**	storage		**10 °C**	storage	

3. Proceed to fluorescence detection after the amplification program is completed.

9. DATA ANALYSIS

Please read the ALA-1/4 Operating Manual before using this kit.

Before the detection run, the required settings of the detector software should be adjusted according to the Guidelines [2].

The detection can be performed within 1 week from the end of the amplification only if the tubes with the amplified product were stored at 2-20ºC.

The PCR kits with end-point detection of fluorescence signals must not be used as methods of quantitative detection of pathogen agent DNA concentrations! It is absolutely forbidden to use the fluorescence signals values obtained during the analysis for quantitative interpretation of results!

9.1 Results interpretation. Variant screen-FEP 2x

Results interpretation for "*HPV*-1" mix *(HPV1 test)*

This mix allows detecting a part of *HPV* HCR genotypes (channel for the FAM fluorophore) and separately identifies genotype 16 (channel for the JOE fluorophore). Correspondently, if positive result is registered in the channel for the FAM fluorophore it indicates "*HPV HCR is detected*" result, if positive result is registered in the channel for the JOE fluorophore it indicates *"HPV genotype 16 is detected"* result.

PCR-mix-1-FEP *HPV*-1 doesn't include Internal Control. So, if negative result is obtained for both channels the complete result of the test (negative or invalid) will be determined by results for "*HPV*-2" mix.

Results interpretation for "*HPV*-2" mix *(HPV2 test)*

This mix allows detecting of another part of *HPV* HCR genotypes (channel for the FAM fluorophore) and Internal Control (channel for the JOE fluorophore). Correspondently, if positive result is registered in the channel for the FAM fluorophore, it indicates *"HPV HCR is detected"* result.

Negative result in the channel for the FAM fluorophore and positive result in the channel for the JOE fluorophore indicate *"HPV HCR is not detected"* result. Negative signal in both, FAM and JOE, channels indicate *"Invalid"* result. However, even if invalid result is defined for "*HPV*-2" mix, total analysis result can be positive in case *HPV* HCR or *HPV* type 16 are found in "*HPV*-1" mix (see table 4).

Table 4

Interpretation of total analysis results

"*HPV*-1" mix (*HPV*1)		**"*HPV*-2" mix (*HPV*2)**		**Result**
FAM (*HPV* HCR)	JOE (*HPV* 16)	FAM (*HPV* HCR)	JOE (IC)	
-	-	-	+	*HPV* HCR is not detected
+ -	-	- +	+	*HPV* HCR is detected
-	+	-	+	*HPV* type 16 is detected
+ - +	+	- + +	+	*HPV* HCR, including type 16 is detected
-	-	-	-	Invalid result
+ -	-	- +	-	*HPV* HCR is detected
-	+	-	-	*HPV* type 16 is detected
+ - +	+	- + +	-	*HPV* HCR including type 16 is detected

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of Amplification as well as for the Negative Control

of Extraction are correct (see Table 5).

Table 5

Results for controls

Control	Stage for control	Result for PCR-mix-1-FEP *HPV*-1	Result for PCR-mix-1-FEP *HPV*-2
C–	DNA extraction	FAM channel *negative* JOE channel *negative*	FAM channel *negative* JOE channel *negative*
NCA	PCR	FAM channel *negative* JOE channel *negative*	FAM channel *negative* JOE channel *negative*
C+	PCR	FAM channel *positive* JOE channel *positive*	FAM channel *positive* JOE channel *positive*

9.2 Results interpretation. <u>Variant screen-FEP 3x</u>

The PCR kit applies a single mixture of primers and probes. Genotypes 31, 35, 39 and 59 are detected in the channel for the ROX fluorophore. Genotypes 16, 18, 33, 45, 52, 58 and 67 are detected in the channel for the FAM fluorophore, Internal Control is detected separately in the channel for the JOE fluorophore.

The result of the analysis is considered reliable only if the results obtained for the Positive and Negative Controls of Amplification as well as for the Negative Control of Extraction are correct (see Table 6).

Table 6

Results for controls

Control	Stage for control	Result of automatic interpretation
C–	DNA extraction	FAM channel *negative* JOE channel *negative* ROX channel *negative*
NCA	PCR	FAM channel *negative* JOE channel *negative* ROX channel *negative*
C+	PCR	FAM channel *positive* JOE channel *positive* ROX channel *positive*

10. TRANSPORTATION

AmpliSens® *HPV* HCR screen-FEP PCR kit should be transported at 2–8 ºC for no longer than 5 days.

11. STABILITY AND STORAGE

All components of the **AmpliSens® *HPV* HCR screen-FEP** PCR kit (except for polymerase (TaqF), PCR-mix-1-FEP *HPV*-1, PCR-mix-1-FEP *HPV*-2, and PCR-mix-1-FEP *HPV* 3x) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *HPV* HCR screen-FEP** PCR kit are stable until the expiration date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF), PCR-mix-1-FEP *HPV*-1, PCR-mix-1-FEP *HPV*-2, and PCR-mix-1-FEP *HPV* 3x are to be stored at the temperature from minus 24 to minus 16 °C.

PCR-mix-1-FEP *HPV*-1, PCR-mix-1-FEP *HPV*-2, and PCR-mix-1-FEP *HPV* 3x are to be kept away from light.

12. SPECIFICATIONS

12.1. Sensitivity

Analytical Sensitivity of **AmpliSens® *HPV* HCR screen-FEP** PCR kit is no less than 1 x 10^3 genome equivalents per 1 ml of sample (GE/ml).

The claimed analytical features of **AmpliSens® *HPV* HCR screen-FEP** PCR kit are guaranteed only when additional reagents kit, DNA-sorb-AM, DNA-sorb-B, or DNA-sorb-C, AmpliSens® DNA-sorb-D (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology"), is used.

12.2. Specificity

The analytical specificity of **AmpliSens® *HPV* HCR screen-FEP** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes were checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

13. REFERENCES

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2012.
2. Guidelines to the **AmpliSens® *HPV* HCR screen-FEP** PCR kit for qualitative detection of high carcinogenic risk (HCR) *human papillomaviruses* (*HPV*) types 16, 18, 31, 33, 35, 39, 45, 52, 58, 59, 67 DNA in the biological material by the polymerase chain reaction (PCR) with end-point hybridization-fluorescence detection, developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

14. QUALITY CONTROL

In compliance with the Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of the **AmpliSens® *HPV* HCR screen-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

15. KEY TO SYMBOLS USED

REF	Catalogue number		Sufficient for
LOT	Batch code		Expiration Date
RUO	Research use only		Consult instructions for use
VER	Version		Keep away from sunlight
	Temperature limitation	**NCA**	Negative control of amplification
	Manufacturer	**C–**	Negative control of extraction
	Date of manufacture	**C+**	Positive control of amplification
	Caution	**IC**	Internal control