



For *in Vitro* Diagnostic Use

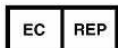
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# AmpliSens<sup>®</sup> HPV 6/11-FEP

PCR kit

## Instruction Manual



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### 1. INTENDED USE.

**AmpliSens® HPV 6/11-FEP** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *Human Papillomavirus* (HPV) types 6 and 11 DNA in the clinical materials (cervical or urethral scrapes) by means of end-point hybridization-fluorescence detection of amplified products.

### 2. PRINCIPLE OF PCR DETECTION.

HPV types 6 and 11 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special HPV 6/11 primers. In end-point PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. Multichannel rotor-type fluorometer is specially designed to detect fluorescent excitation from the fluorophores in a reaction mix after PCR. **Fluorescent End-Point PCR (FEP-PCR)** allows the detection of accumulating product without re-opening of the reaction tubes after the PCR run. **AmpliSens® HPV 6/11-FEP** PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) activates by heating at 95°C for 15 min.

The test is based on simultaneous amplifying (multiplex PCR) of DNA fragments of HPV and a fragment of  $\beta$ -globin gene which is used as an internal endogenous control. The test is running in a single tube.

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, not only does endogenous internal control allow to monitor stages of the test (DNA extraction and PCR conducting) but also to assess the adequacy of clinical material collection and storage. If the quantity of epithelium in the specimen is insufficient,  $\beta$ -globin gene amplification signal of will be weak.

HPV genotypes 6 and 11 are low carcinogenic risk viruses that are not associated with cervical carcinoma. Low-risk HPV types have a productive effect on cells. These viruses are responsible for onset of genital warts (genital pointed condyloma), recurrent respiratory throat papillomatosis; and more than 95% of these pathologies associated with genotypes 6 and 11.

Necessary to mention, that genotypes 6 and 11 are the part of quadrivalent vaccine which protects from HPV 6/11/16/18 infections. Therefore, detection of HPV types 6 and 11 can help in evaluating of the efficacy of vaccinal prevention from cervical cancer and benign genital tumors.

### 3. CONTENT.

**AmpliSens® HPV 6/11-FEP** PCR kit is produced in 1 form:

AmpliSens® HPV 6/11-FEP PCR kit, **REF** V11-FEP-CE.

AmpliSens® HPV 6/11-FEP PCR kit includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1-FEP/FRT HPV 6/11	colorless, clear liquid	0.6	2 tubes
PCR-mix-2-FRT	colorless, clear liquid	0.3	2 tubes
Polymerase (TaqF)	colorless, clear liquid	0.03	2 tubes
PCR-mix-Background HPV	colorless, clear liquid	0.8	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 tube
Positive Control DNA HPV types 6, 11 and human DNA (C+)	colorless, clear liquid	0.2	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube
Negative Control (C-)*	colorless, clear liquid	1.2	1 tube

\* must be used in the isolation procedure as Negative Control of Extraction.

**AmpliSens® HPV 6/11-FEP** PCR kit is intended for 120 reactions, including controls.

### 4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200  $\mu$ l).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Gradient Palm Cyclor (Corbett Research, Australia), GeneAmp PCR System 2700 (Applied Biosystems,USA), Uno-2 (Biometra,Germany), Terzik (DNA-Technology, Russia).
- Fluorometer ALA-1/4 (Biosan, Latvia) or equivalent instrument.
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity (for example, Axygen, USA).
- Refrigerator for 2–8 °C.
- Deep-freezer with temperature below minus 16 °C.
- Waste bin for used tips.

### 5. GENERAL PRECAUTIONS.

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- Store extracted positive material (samples, controls and amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly at room temperature before starting an assay.

- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, 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 a kit after its expiration date.
- Dispose all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5 % sodium hypochlorite, or other suitable disinfectant.
- Avoid contact with the skin, eyes and mucose membranes. If skin, eyes and mucose membranes contact immediately flush with water, seek medical attention
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be unidirectional; it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment and reagents to the area in which 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.



In detail, sampling of biological materials for PCR-analysis, transportation and storage are described in handbook of the manufacture [1]. It is recommended that this handbook is read before starting the work.

**AmpliSens® HPV 6/11-FEP** PCR kit is intended to analyze DNA extracted with DNA isolation kits from

– cervical or urethral scrapes

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

**Method 1** - use the sampling kit which includes one/two cervical cytobrushes and 2 ml tube with 0.5 ml of transport medium with mucolytic "TSM" **REF** 953.

Endocervical epithelial scrape, obtained with first cytobrush and/or exocervical epithelial scrape obtained with second cytobrush should be placed into the tube with transport media. Break the effective part of the cytobrush with the sample at the score mark and leave it in the tube.

**Method 2** - use "Digene" (USA) sampling kit, which contains cervical cytobrush and 1.0 ml tube with "Digene" transport medium.

Endocervical epithelial scrape obtained with cytobrush should be placed into the tube with "Digene" transport medium.

**Method 3** - use the sampling kit, which contains combined gynecological probe for simultaneous obtaining of epithelial cells from endo-/exocervix and 5 ml tube with 2.0 ml of transport medium with mucolytic "TSM" **REF** 953.

Place endocervical and exocervical epithelial scrapes into the tube with transport medium. Break the effective part of the cytobrush with the sample at the score mark and leave it in the tube.

**Method 4** - use "CytoScreen" (Italy) or "PreservCyt" (USA) sampling kits which contain combined gynecological probe for simultaneous obtaining of epithelium from endo-/exocervix and a vial with transport-fixation medium.

Place endocervical and exocervical epithelial scrapes into the tube with transport-fixation medium. Break the effective part of the cytobrush with the sample at the score mark and leave it in the vial.

**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 "TSM" **REF** 953.



Only one freeze-thaw cycle of clinical material is allowed.

## 7. PROTOCOL.

### 7.1. DNA Isolation

It's recommended that the following nucleic acid extraction kits are used:

- "DNA-sorb-AM", **REF** K1-12-100-CE (for clinical material obtained by methods 1, 2, 3);
- "DNA-sorb-B", **REF** K1-2-100-CE (for clinical material obtained by methods 1, 2, 3);
- "DNA-sorb-C", **REF** K1-6-50-CE (for biopsy of mucous)



Please carry out the DNA isolation according to the manufacturer instruction.

### 7.2. Preparing the PCR.

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

#### 7.2.1 Preparing tubes for PCR.

1. Prepare the mix of PCR-mix-2-FRT and polymerase (TaqF). To do this, transfer the whole amount of the tube with **polymerase (TaqF) (30 µl)** to the tube with **PCR-mix-2-FRT (300 µl)**. Vortex carefully to avoid foaming. Indicate the mix preparation time on the tube.



Prepared mix is intended for 60 samples.  
Store between 2 and 8 °C for 3 months and use as appropriate.

2. Prepare the reaction mix (see Appendix 1). Add two control reactions (negative and positive controls) and one spare reaction when calculating the reaction mix volume.

Each PCR reaction requires:

- **10 µl of PCR-mix-1-FEP/FRT HPV 6/11**
- **5 µl of mix of PCR-mix-2-FRT and polymerase (TaqF).**

3. Collect the required number of the PCR tubes for amplification of clinical and control samples.

Transfer **15 µl** of prepared reaction mix to each tube.

4. Add above 1 drop of **mineral oil for PCR** (about 25 µl).

5. Prepare 2 control **Background** samples:

- If one of the **recommended DNA extraction kits** has been applied, add **10 µl of PCR-mix-FEP/FRT HPV 6/11** and **15 µl of PCR-mix-Background HPV** per each Background tube. Add above 1 drop of **mineral oil for PCR**.
  - If **another DNA extraction method** has been applied, add **10 µl of PCR-mix-FEP/FRT HPV 6/11**, **5 µl of PCR-mix-2-FRT**, and **10 µl of Negative Control** of extraction per each Background tube.
- Add above 1 drop of **mineral oil for PCR**.



Background samples, that have been thermocycled once, can be used for further runs without thermo cycling. Multiple use of Background samples is permitted in case of application of the same lot of PCR kit; same extraction kit and same tube type. Store the Background tubes from 2 to 20 °C for up to 1 month. Keep away from light.

6. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared tubes.

7. Carry out control amplification reactions:

- NCA** - add **10 µl** of **DNA-buffer** to the tube labeled NCA (Negative Control of Amplification).
- C+** - Add **10 µl** of **Positive Control DNA HPV types 6, 11 and human DNA** to the tube labeled C+ (Positive Control of Amplification).

### 7.2.2 Amplification.

Run the following program on the thermocycler (see Table 1). When the temperature reaches 95 °C (pause regimen), insert tubes to cells of amplifier and press the button to continue.

It is recommended that drops are removed from walls of the tubes by short vortex (1–3 sec) before placing in the thermocycler.

Table 1

Programming thermocyclers for HPV 6, 11 DNA amplification

step	Thermocyclers with active temperature adjustment:					
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems); "Palm-Cycler" (Corbett Research); "Maxygene" (Axygen)		
	temperature	time	cycles	temperature	time	cycles
1	95 °C	15 min	1	95 °C	15 min	1
2	95 °C	10 sec	45	95 °C	20 sec	45
	60 °C	40 sec		60 °C	45 sec	
3	72 °C	1 min	1	72 °C	1 min	1
4	10 °C	storage		10 °C	storage	



If this PCR kit is used simultaneously with AmpliSens® HPV HCR screen-FL PCR kit then the unified amplification program can be applied (table 2).

Programming thermocyclers for HPV DNA amplification

step	Thermocyclers with active temperature adjustment:									Other thermocyclers		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (Applied Biosystems)			"Gradient Palm Cycler" (Corbett Research); "Maxygene" (Axygen)			temperature	time	cycles
	temperature	time	cycles	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	95 °C	15 min	1
2	93 °C	5 sec	20	95 °C	10 sec	50	93 °C	5 sec	50	95 °C	25 sec	50
	59 °C	5 sec		59 °C	20 sec		59 °C	10 sec		59 °C	25 sec	
	72 °C	5 sec		72 °C	10 sec		72 °C	5 sec		72 °C	25 sec	
3	93 °C	2 sec	30	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
	59 °C	10 sec		95 °C	20 sec	1	95 °C	20 sec	1	95 °C	20 sec	1
4	10 °C	storage		10 °C	storage		10 °C	storage		10 °C	storage	



Detection can be performed within 1 week from the end of amplification in case the tubes were stored at temperature from 2 to 20 °C.

### 8. DATA ANALYSIS.

Detection is conducted on ALA-1/4 fluorescence detector (Biosan, Latvia).



Please read Aladin Operating Manual before use of this kit.

Program the detector according to manufacturer's manual and Appendix 2.

### Results interpretation

- When the analysis is complete the results are automatically shown in the table in the manner of following indications:
  - pos** – positive result;
  - neg** – negative result;
  - eq** – equivocal result (signal is in grey zone);
  - nd** – invalid result (specific signal and IC signal are absent in the sample).
- Result of the analysis is considered reliable only if both Positive and Negative Controls of amplification as well as Negative Control of extraction are passed (Table 3).

Table 3

Results for controls

Control	Stage for control	Result of automatic interpretation			Interpretation
		FAM channel	HEX channel	ROX channel	
C-	DNA isolation	HPV 6 - neg	HPV 11- neg	IC-	OK
NCA	Amplification	HPV 6 - neg	HPV 11 - neg	IC-	OK
C+	Amplification	HPV 6 - pos	HPV 11 - pos	IC+	OK

This PCR kit allows to detect HPV genotype 6 (FAM channel) and 11 (HEX channel) **separately**.

1. Positive signal on FAM channel means that HPV genotype 6 is detected.
2. Positive signal on HEX channel means that HPV genotype 11 is detected.
3. Negative result on FAM and HEX channels along with positive signal on ROX channel means that *Human Papillomaviruses* are not detected.

## 9. TROUBLESHOOTING.

If analysis results are not obtained as per the following examples:

- If positive signal is absent in positive control of PCR (C+), check programming of the thermocycler (see 7.2.2.), storage conditions, and the expiration date of the reagents and repeat PCR reaction once again for all samples.
- Negative signal on all channels (except for C-) indicates that the result is invalid. All samples should be examined repeatedly starting from the extraction stage. If invalid result is obtained once more, it is necessary to collect new clinical material.
- Positive signal in negative controls (C- or NCA) indicates the reagents or samples contamination. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests, and also to take measures to detect and eliminate the source of contamination.

If you have any further questions or if you encounter problems, please contact our Authorized representative in the European Community.

## 10. STABILITY AND STORAGE.

All components of the **AmpliSens® HPV 6/11-FEP** PCR kit (except for polymerase (TaqF), PCR-mix-1-FEP/FRT HPV 6/11, and PCR-mix-2-FRT) are to be stored between 2°C and 8°C, when not in use. They also must be stable until the expiry date stated on the label.



Polymerase (TaqF), PCR-mix-1-FEP/FRT HPV 6/11, and PCR-mix-2-FRT are to be stored at not more than minus 16 °C.

## 11. SPECIFICATIONS.

### 11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® HPV 6/11-FEP** PCR kit is no less than  $1 \times 10^3$  genome equivalents per 1 ml of sample (GE/ml).



The claimed analytical features of **AmpliSens® HPV 6/11-FEP** PCR kit are guaranteed only when additional reagents kits, “DNA-sorb-AM”, “DNA-sorb-B”, or “DNA-sorb-C”(manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

### 11.2. Specificity.

Specificity of **HPV 6/11-FEP** PCR kit is assured by selection of specific primers and probes, as well as the selection of strict reaction conditions. The primers and probes were checked for possible homologies to all in gene banks published sequences by sequence comparison analysis. Specificity of **AmpliSens® HPV 6/11-FEP** PCR kit was confirmed in laboratory clinical trials.

















## 12. REFERENCES.

1. Handbook “Sampling, transportation, storage of clinical material for PCR diagnostics”, developed by Federal State Institution of Science “Central Research Institute of Epidemiology” of Federal Service for Surveillance on Consumers’ Rights Protection and Human Well-Being, Moscow, 2008.

## 13. QUALITY CONTROL.

In compliance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 – certified Quality Management System, each lot of **AmpliSens® HPV 6/11-FEP** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

## 14. EXPLANATION OF SYMBOLS

	Manufacturer		Temperature limitation
	Use by		Batch code
	For <i>in Vitro</i> Diagnostic Use		Version
	Catalogue number		Internal Control complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with iQ5, iQ iCycler
	Positive control		Negative control