



For *in Vitro* Diagnostic Use

## TABLE OF CONTENTS

1. INTENDED USE.....	3
2. PRINCIPLE OF PCR DETECTION.....	3
3. CONTENT.....	4
4. ADDITIONAL REQUIREMENTS.....	5
5. GENERAL PRECAUTIONS.....	5
6. SAMPLING AND HANDLING.....	6
7. PROTOCOL.....	7
8. DATA ANALYSIS.....	8
9. TROUBLESHOOTING.....	9
10. STABILITY AND STORAGE.....	10
11. SPECIFICATIONS.....	10
12. REFERENCES.....	10
13. QUALITY CONTROL.....	10
14. EXPLANATION OF SYMBOLS.....	11

# AmpliSens<sup>®</sup> HPV HCR screen-EPh PCR kit

## Instruction Manual

### AmpliSens<sup>®</sup>

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## 1. INTENDED USE.

**AmpliSens® HPV HCR screen-EPh** PCR kit is an in vitro nucleic acid amplification test for qualitative detection of *Human Papillomavirus* of high carcinogenic risk types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70 DNA in the clinical material (cervical or urethral scrapes) by using electrophoretic detection of the amplified products in agarose gel.

## 2. PRINCIPLE OF PCR DETECTION.

HPV HCR detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen cDNA specific region using special HPV HCR primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® HPV HCR screen-EPh** PCR kit is able to detect HPV DNA of three main phylogenetic groups: A7, A6, A9 which include types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70. These types have high transformation activity. They are responsible for more than 97% of severe cervical dysplasia and cervical cancer. **AmpliSens® HPV HCR screen-EPh** PCR kit contains the internal endogenous control (fragment of  $\beta$ -globine gene). **AmpliSens® HPV HCR screen-EPh** 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 wax layer or chemically modified polymerase (TaqF). The wax melting and reaction mix component occurs only at 95°C. Chemically modified polymerase (TaqF) activates by heating at 95°C for 15 min. The test is based on simultaneous amplifying (multiplex PCR) of E1-E2 genes fragments of three HPV groups and a fragment of  $\beta$ -globine gene which is used as internal endogenous control. Test is running in a single tube. All amplifying products have similar length.

### Detection of clinically significant virus quantity by using of **AmpliSens® HPV HCR screen-EPh** 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 clinical material is provided. Clinical investigations done on model clinical 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 0.6 ml of transport media).
- DNA extraction (“DNA-sorb-A” or “DNA-sorb-AM” were used).

- 100x dilution of obtained DNA in TE-buffer.
- PCR-test.

Clinical 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-EPh** 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 clinical material is necessary.

## 3. CONTENT.

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

AmpliSens® HPV HCR screen-EPh PCR kit variant 50 F, **REF** V31-50F-CE.

AmpliSens® HPV HCR screen-EPh PCR kit variant 100 F, **REF** V31-100F-CE.

**AmpliSens® HPV HCR screen -EPh** PCR kit variant 50 F or variant 100 F includes:

Reagent	Description	variant 50 F		variant 100 F	
		Volume (ml)	Quantity	Volume (ml)	Quantity
<b>PCR-mix -1 HPV HCR screen</b>	colorless, clear liquid	0.3	1 tube	0.3	2 tubes
<b>2.5x PCR-buffer blue</b>	blue clear liquid	0.6	1 tube	1.15	1 tube
<b>Polymerase (TaqF)</b>	colorless, clear liquid	0.03	1 tube	0.06	1 tube
<b>Mineral oil for PCR</b>	colorless viscous liquid	2.0	1 dropper bottle	4.0	1 dropper bottle
<b>Positive Control DNA HPV types 31, 39, 56 and DNA human</b>	colorless, clear liquid	0.2	1 tube	0.2	1 tube
<b>Positive Control DNA human</b>	colorless, clear liquid	0.2	1 tube	0.2	1 tube
<b>TE-buffer</b>	colorless, clear liquid	5.0	2 tubes	5.0	4 tubes
<b>Negative Control (C-)*</b>	colorless, clear liquid	1.2	1 tube	1.2	1 tube

\* must be used in the isolation procedure as Negative Control of Extraction (see “DNA-sorb-AM”, **REF** K1-12-100-CE, or “DNA-sorb-B”, **REF** K1-2-100-CE or “DNA-sorb-C”, **REF** K1-6-50-CE protocols).

AmpliSens® HPV HCR screen-EPh PCR kit variant 50 F is intended for 55 reactions, including controls.

AmpliSens® HPV HCR screen-EPh PCR kit variant 100 F is intended for 110 reactions, including

controls.

#### 4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Agarose gel detection kit.
- Disposable powder-free gloves and laboratory coat.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers (up to 200 µl).
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Personal thermocyclers (for example, Terzik (DNA-Technology), GeneAmp PCR System 2400, GeneAmp PCR System 2700 (Applied Biosystems), T-personal (Biometra), PTC-100 (MJ Research)).
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, "Axygen", USA).
- Refrigerator with temperature between 2 and 8 °C.
- Deep-freezer with temperature not more than minus16°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 and handle amplicons away from all other reagents.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, protect eyes while samples and reagents handling. Thoroughly wash hands afterward.
- 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 of all samples and unused reagents in compliance with local authorities requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance 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 mucosa. If skin, eyes and mucosa 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 one directional, 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.



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

AmpliSens<sup>®</sup> HPV HCR screen-EPh PCR kit is intended for analysis DNA extracted with DNA isolation kits from:

- *Cervical or urethral scrapes.*

##### 6.1. *Cervical or urethral scrapes.*

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

**Method I** - use the sampling kit which includes one/two cervical cytobrushes and 2 ml tube with 0.5 ml of transport media "TSM".

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 II** - use "Digene" (USA) sampling kit, which contain cervical cytobrush and 1.0 ml tube with "Digene" transport media.

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

**Method III** - use the sampling kit, which contain combined gynecological probe for simultaneous obtaining of epithelial cells from endo-/exocervix and 5 ml tube with 2.0 ml of transport media "TSM".

Place endocervical and exocervical epithelial scrapes 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 IV** - 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 media.

Place endocervical and exocervical epithelial scrapes into the tube with transport-fixation media. 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 media "TSM".



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

## 7. PROTOCOL.

### 7.1. DNA Isolation

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

- "DNA-sorb-AM", **REF** K1-12-100-CE.
- "DNA-sorb-B", **REF** K1-2-100-CE.
- "DNA-sorb-C", **REF** K1-6-50-CE.



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

### 7.2. Preparing the PCR.

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

#### 7.2.1 Preparing tubes for PCR.

1. Prepare reaction mix for N reactions:

**5\*(N+1) µl of PCR-mix-1 HPV HCR screen**

**10\*(N+1) µl of 2.5x PCR-buffer blue**

**0.5\*(N+1) µl of polymerase (TaqF)**



Store prepared reaction mix for up to 2 hours.



When calculating reaction mix volume of additional reactions three controls and one extra reaction should be included.

2. Spin the tube with the reaction mix. Add 15 µl of the reaction mix into PCR tubes.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). This step could be omitted if thermocycler with constant-temperature closure is used.
4. Using tips with aerosol barrier add **10 µl of DNA samples** obtained from clinical or control samples at the DNA extraction stage, under oil or directly on the level of oil. (in case of *clinically significant quantity of HPV HCR format* use 100x dilution of obtained DNA samples).

5. Carry out the control amplification reactions:

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

HPV- - Add 10 µl of **Positive Control DNA human** to the tube for Positive Control of human DNA Amplification.

HPV+ - Add 10 µl of **Positive Control DNA HPV types 31, 39, 56 and human DNA** to the tube for 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 button to continue.

It is recommended to sediment drops from walls of tubes by short vortex (1–3 sec) before their insertion in thermocycler.

Table 1

#### Programming thermocyclers for DNA amplification of DNA of HPV HCR

**types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70**

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"Terzik" (DNA-Technology)			"GeneAmp PCR System 2400" (Perkin Elmer), "GeneAmp PCR System 2700" (Applied Biosystems)			"T-personal" (Biometra), "PTC-100" (MJ Research)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
1	95 °C	900 sec	1	95 °C	900 sec	1	95 °C	900sec	1
2	95 °C	10 sec	42	95 °C	15 sec	42	95 °C	30 sec	42
	63 °C	20 sec		63 °C	30 sec		65 °C	40 sec	
	72 °C	20 sec		72 °C	30 sec		72 °C	40 sec	
3	72 °C	60 sec	1	72 °C	60 sec	1	71 °C	60 sec	1

Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment - 1 h 50 min.

After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

Analysis of amplification products is performed by separation of DNA fragments in agarose gel.

The amplified samples can be stored for 16 h at room temperature, for 1 week at 2 – 8 °C (be sure to warm the samples to room temperature before running electrophoresis).

## 8. DATA ANALYSIS.

It's recommended to use the following detection agarose kit:

- "EPH" variant 200, **REF** K5-200-CE.

Analysis of results is based on the presence or absence of specific bands of amplified DNA in agarose gel (1.7%).

PCR-mix -1 HPV HCR screen includes primers for amplification of 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70 types DNA fragments as well as human genome DNA fragment ( $\beta$ -globine gene).

The length of specific amplified DNA fragments is:

- Internal Control (fragment of  $\beta$ -globine gene ) - 723 bp
- HPV types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70 - from 267 to 325



Put the protective mask or use the glass filter while watching and photographing the gel

### 8.1. Results interpretation.

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel		Interpretation
		267-325 bp	723 bp	
C-	DNA isolation	No	No	OK
NCA	Amplification	No	No	OK
HPV-	Amplification	No	Yes	OK
HPV+	Amplification	Yes	Yes	OK

- The sample is considered to be positive for HPV HCR DNA if the band at the level from 267 to 325 bp is present in agarose gel regardless of the band of Internal Control (723 bp).
- The sample is considered to be negative for HPV HCR DNA if the band of 723 bp is present.

Besides specific bands the indistinct washed-out bands of primer-dimers may be seen in lanes, they are situated lower than level of 100 bp of nucleotide pairs.

### 9. TROUBLESHOOTING.

Analysis results are not obtained as per the following examples:

- If results of control points analysis do not correspond to the listed above (Table 2), then the tests should be repeated. Discard any reagents that may be suspect.
- If in lane corresponding to a clinical sample the bands of Internal Control (723 bp) is absent insufficient quantity of clinical material or mistakes in clinical processing, DNA extraction, or PCR conducting can be suggested.
- If in lines nonspecific bands at different levels are presented, it may be caused by lack of «hot start» or false temperature regimen in thermocycler.
- If in lanes corresponding to negative control (NCA, C-) specific bands appear, it means that reagents or samples contamination has taken place. In such cases results of analysis must be considered as irrelevant. Test analysis must be repeated and measures for detecting contamination source must be undertaken.

### 10. STABILITY AND STORAGE.

All components of the **AmpliSens® HPV HCR screen-EPh** PCR kit are to be stored at the temperature between 2 °C and 8 °C (except for polymerase TaqF) when not in use. All components of the PCR kit are to be stable until labeled expiration date.



Polymerase (TaqF) is to be stored at the temperature not more than minus 16 °C.

### 11. SPECIFICATIONS.

#### 11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® HPV HCR screen-EPh** PCR kit is no less than  $5 \times 10^3$  genome equivalents per 1 ml of sample (GE/ml) (types 16, 18, 31, 35, 39, 45, 52, 56, 59, 66, 70) and  $2,5 \times 10^4$  GE/ml (types 33, 58).



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

#### 11.2. Specificity.

Specificity of **AmpliSens® HPV HCR screen-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.












### 12. REFERENCES.

1. Manual “Sampling, transportation and 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 Total Quality Management System, each lot of **AmpliSens® HPV HCR screen-EPh** PCR kit is 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		Contains sufficient for <N> tests
	Authorised representative in the European Community.		Consult instructions for use
	Caution, consult accompanying documents	<b>NCA</b>	Negative Control of Amplification
<b>HPV-</b>	Positive Control of human DNA Amplification	<b>HPV+</b>	Positive Control of Amplification
<b>C-</b>	Negative Control of Extraction		