

For *In Vitro* Diagnostic Use

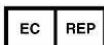
# AmpliSens® HPV 16/18-EPh PCR kit

## Instruction Manual

# AmpliSens®

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Ecoli s.r.o., Studenohorská 12  
841 03 Bratislava 47  
Slovak Republic  
Tel.: +421 2 6478 9336  
Fax: +421 2 6478 9040  
ecoli@ecoli.sk  
[www.ecoli.sk](http://www.ecoli.sk)  
[www.pcrdiagnostics.eu](http://www.pcrdiagnostics.eu)



Federal State Institution of Science  
“Central Research Institute of Epidemiology”  
3A Novogireevskaya Street  
Moscow 111123  
Russia

### 1. INTENDED USE

**AmpliSens® HPV 16/18-EPh PCR kit** is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *Human Papillomavirus* types 16 and 18 in the clinical material (cervical or urethral scrapes) by means of detection of the amplified products by agarose gel electrophoresis. **AmpliSens® HPV 16/18-EPh PCR kit** is considered auxiliary for PAP infections examination and allows differentiating of two most oncogenic types of the virus. For primary analysis we recommend using **AmpliSens HPV HCR screen-EPh** (electrophoretic detection), **AmpliSens HPV HCR screen-FL**, or **AmpliSens HPV HCR screen-titer-FL** (hybridization fluorescent detection) **PCR kits** that allow detection of wide range (11-14) of highly oncogenic types of Human Papillomavirus.

### 2. PRINCIPLE OF PCR ASSAY

*Human papillomavirus* types 16 and 18 detection by the polymerase chain reaction (PCR) is based on the amplification of specific region of DNA of pathogen genome using specific HPV 16/18 primers. After PCR the amplified product is detected in agarose gel.

**AmpliSens® HPV 16/18-EPh PCR kit** is based on simultaneous amplifying (multiplex-PCR) of DNA fragments of human papillomavirus and  $\beta$ -globine gene which is used as endogenous internal control. PCR test for HPV types 16 and 18 DNA is performed in a single tube. DNA-target selected as endogenous internal control is the fragment of human genome and must be present in specimen in sufficient quantity equivalent to that of cells in the sample (no less than  $10^3$ - $10^5$  genomes). Therefore, not only does endogenous internal control allow monitoring of stages of the test (DNA extraction and PCR conducting) but also evaluating of the adequacy of clinical material collection and storage. If there is not sufficient quantity of epithelial cells in specimen, internal control band will be absent in agarose gel.

**AmpliSens® HPV 6/11-EPh PCR kit** uses “hot-start”, that is guaranteed by separation of nucleotides and Taq-polymerase by wax layer. Melting of wax and mix of reaction components occur only at 95°C, which greatly diminish frequency of nonspecifically primed reactions. In **AmpliSens® HPV 6/11-EPh PCR kit variant 50 F** “hot start” is ensured by application of chemically modified polymerase (TaqF) that activates when warmed at 95°C for 15 min.

### 3. CONTENTS OF THE KIT

**AmpliSens® HPV 16/18-EPh PCR kit** is produced in 4 forms:

**AmpliSens® HPV 16/18-EPh PCR kit variant 100 R** (vials 0.5 ml), **REF** V12-100-R0,5-CE.

**AmpliSens® HPV 16/18-EPh PCR kit variant 100 R** (vials 0.2 ml), **REF** V12-100-R0,2-CE.

**AmpliSens® HPV 16/18-EPh PCR kit variant 200**, **REF** V12-200.

**AmpliSens® HPV 16/18-EPh PCR kit variant 100 F**, **REF** V12-100 F.

**AmpliSens® HPV 16/18-EPh PCR kit variant 100 R** or variant 200 includes:

Reagent	Description	variant 100 R		variant 200	
		Volume (ml)	Amount	Volume (ml)	Amount
PCR-mix -1-R HPV 16/18 ready-to-use single-dose test tubes ( <i>under wax</i> )	colorless, clear fluid	0.005	110 vials of 0.5 or 0.2 ml	---	---
PCR-mix-1 HPV 16/18	colorless, clear fluid	---	---	1.1	1 vial
PCR-mix-2 blue	clear fluid of blue color	1.2	1 vial	1.2	2 vials
Wax for PCR	hard white matter	---	---	1.7	2 vials
Mineral oil for PCR	colorless viscous fluid	4.0	1 vial	8.0	1 vial
Positive Control DNA HPV types 16,18 and human DNA (C+) (C+)	colorless, clear fluid	0.2	1 vial	0.2	1 vial
DNA-buffer	colorless, clear fluid	0.5	1 vial	0.5	1 vial
Negative Control (C-)*	colorless, clear fluid	1.2	1 vial	1.2	1 vial

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

(see “DNA-sorb-A”, [REF](#) K1-1-100-CE, or “DNA-sorb-B”, [REF](#) K1-2-100-CE, or “DNA-sorb-AM” [REF](#) K1-7-100-CE protocols)

AmpliSens® HPV 16/18-EPh PCR kit variant 100 R is sufficient for 110 reactions, including controls.

AmpliSens® HPV 16/18-EPh PCR kit variant 200 is sufficient for 220 reactions, including controls.

**AmpliSens® HPV 16/18-EPh PCR kit variant 100 F** includes:

Reagent	Description	Volume (ml)	Amount
PCR-mix-1 HPV 16/18	colorless, clear fluid	1.2	1 vial
2.5x PCR-buffer blue	clear fluid of blue color	1.15	1 vial
Polymerase (TaqF)	colorless, clear fluid	0.06	1 vial
Mineral oil for PCR	colorless viscous fluid	4.0	1 vial
Positive Control DNA HPV types 16, 18 and human DNA (C+)	colorless, clear fluid	0.2	1 vial
DNA-buffer	colorless, clear fluid	0.5	1 vial
Negative Control (C-)*	colorless, clear fluid	1.2	1 vial

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

(see “DNA-sorb-A”, [REF](#) K1-1-100-CE, or “DNA-sorb-B”, [REF](#) K1-2-100-CE, or “DNA-sorb-AM” [REF](#) K1-7-100-CE protocols)

AmpliSens® HPV 16/18-EPh PCR kit variant 100 F is sufficient for 110 reactions, including controls.

#### 4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES

- Disposable powder-free gloves
- DNA isolation kit
- Detection agarose kit
- Pipettes (adjustable)
- Sterile pipette tips with aerosol filters ( up to 200 µl)
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- PCR box
- Personal thermocyclers
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8 °C with deep-freezer with temperature no less than –16°C
- Reservoir for disposed tips

#### 5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and put the new tip for every procedure.
- Store and handle amplicons separately from all other reagents.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. 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 specimens and unused reagents in accordance with local regulations.
- Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucose membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
- 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.
- Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.



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

#### 6. SPECIMEN COLLECTION AND HANDLING

AmpliSens® HPV 16/18-EPh PCR kit is intended to analyze 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:

**First method** — uses the specimen collection kit including one/two cervical cytobrushes and 2 ml tube with 0.5 ml of transport media [REF](#) V12-100-RO,5-CE (R0,2-CE); V12-200; V12-100F

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“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.

**Second method** — use “Digene” (USA) collection kit, containing 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.

**Third method** — use sample collection kit, containing 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.

**Fourth method** — use “CytoScreen” (Italy) or “PreservCyt” (USA) samples collection kits containing 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”.

Storage of native and treated samples.

- at room temperature for 5 days
- at 2 °C – 8 °C for 20 days;
- at minus 16 °C for 1 year;



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

#### 7. PROTOCOL

##### 7.1. DNA Isolation

Different manufacturers offer DNA isolation kits. We recommend following nucleic acid extraction kits:

- “DNA-sorb-A”, [REF](#) K1-1-100-CE.
- “DNA-sorb-AM”, [REF](#) K1-7-100-CE.
- “DNA-sorb-B”, [REF](#) K1-2-100-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

**AmpliSens® HPV 6/11-EPh PCR kit variant 100 R** or variant 200



When using AmpliSens® HPV 16/18-EPh PCR kit variant 100 R steps 1 and 2 should be omitted.

1. Place the tube with **Wax for PCR** into the heat block at 95 °C to melt the wax completely.
2. Prepare required quantity of the PCR tubes. Pipette 5 µl of **PCR-mix-1 HPV 16/18** into the bottom of each tube. Add a drop (about 10-15 µl) of melted wax above, so it covers completely the liquid, close the caps and mark each tube. The prepared tubes could be stored at 2 – 8 °C during 1 week.
3. Collect the required quantity of tubes prepared as describes above or tubes with **PCR-mix-1-R HPV 16/18** with wax for amplification of DNA of study and control samples.
4. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, so that it wouldn't fall under the wax and mix with reagents in the tube.
5. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.

[REF](#) V12-100-RO,5-CE (R0,2-CE); V12-200; V12-100F

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## AmpliSens® HPV 16/18-EPh PCR kit variant 100 F

1. Prepare reaction mix for N reactions:

**5•(N+1) µl of PCR-mix-1 HPV 16/18**

**9.5•(N+1) µl of 2.5x PCR-buffer blue**

**0.5•(N+1) µl of polymerase (TaqF)**



When calculating reaction mix volume additional reactions should be included: three controls and one reserve reaction.



It is permitted to mix 2.5x PCR-buffer blue and polymerase (TaqF) in advance. Transfer polymerase (TaqF) (0.06 ml) into the tube with 2.5x PCR-buffer blue (1.15 ml). Spin the tube carefully. Mark the tube indicating the date of preparation. Prepared mix is sufficient for 60 reactions. Stored at 2 – 8 °C for up to 3 months.

2. Spin the tube with the reaction mix. Pipette 15 µl of the reaction mix into PCR tubes.

3. Add above 1 drop of **mineral oil for PCR**. When using thermocycler with heating cover this step could be omitted.

### 7.2.2 Amplification

Use prepared tubes for PCR. Under or immediately above the level of oil, using tips with aerosol barrier, **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction.

Perform **control amplification reactions**:

NCA	Add 10 µl of <b>DNA-buffer</b> to the tube for Negative Control of Amplification (NCA).
C+	Add 10 µl of <b>Positive Control DNA HPV types 16, 18 and human DNA</b> the tube for Positive Control of Amplification.

Run the following program on the thermocycler (see table 1 or table 2). When the temperature will reach 95 °C (pause regimen), insert tubes into 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 HPV types 16, 18 (variant 100 R or variant 200)

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"GeneAmp PCR System 2400" (ABI), "Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (ABI), "Gradient Palm Cycler" (Corbett)			"Biomtra", "MiniCycler", "PTC-100" (MJ Research)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	5 min	1	95 °C	5 min	1	95 °C	5 min	1
2	95 °C	10 sec	42	95 °C	15 sec	42	95 °C	1 min	42
	65 °C	10 sec		65 °C	25 sec		65 °C	1 min	
	72 °C	10 sec		72 °C	25 sec		72 °C	1 min	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	4 °C	storage		4 °C	storage		10 °C	storage	

Table 2. Programming thermocyclers for DNA amplification of HPV types 16, 18 (variant 100 F)

Step	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"GeneAmp PCR System 2400" (ABI), "Terzik" (DNA-Technology)			"GeneAmp PCR System 2700" (ABI), "Gradient Palm Cycler" (Corbett)			"Biomtra", "MiniCycler", "PTC-100" (MJ Research)		
	Temperature	Time	Cycles	Temperature	Time	Cycles	Temperature	Time	Cycles
0	95 °C	pause		95 °C	pause		95 °C	pause	
1	95 °C	15 min	1	95 °C	15 min	1	95 °C	15min	1
2	95 °C	10 sec	42	95 °C	15 sec	42	95 °C	1 min	42
	65 °C	10 sec		65 °C	25 sec		65 °C	1 min	
	72 °C	10 sec		72 °C	25 sec		72 °C	1 min	
3	72 °C	1 min	1	72 °C	1 min	1	72 °C	1 min	1
4	4 °C	storage		4 °C	storage		10 °C	storage	

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

We recommend 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%). The length of specific amplified DNA fragments is:

- **HPV type 16** **325 bp**
- **HPV type 18** **425 bp**
- Internal Control (fragment of β-globine gene) - 723 bp



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

### 8.1. Results interpretation

Table 3.

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel			Interpretation
		325 bp	425 bp	723 bp	
C-	DNA extraction	No	No	No	Valid result
NCA	Amplification	No	No	No	Valid result
C+	Amplification	Yes	Yes	Yes	Valid result

- The sample is considered to be positive for HPV types 16 and 18 DNA if the bands of 325 bp and 425 bp are present in agarose gel regardless of the band of Internal Control (723 bp).

- The sample is considered to be negative for HPV types 16 and 18 DNA if the bands of 325 bp and 425 bp are absent and 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

Results of analysis are not being registered in the following cases:

- If results of control points analysis do not correspond to the listed above (Table 3), then the tests are to be re-installed. Discard any reagents that may be suspect.

- If in lane corresponding to a clinical sample the bands of Internal Control (723 bp) is absent it can suggest about insufficient quantity of clinical material or mistakes in clinical processing, DNA extraction, or PCR conducting.

- 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 lane corresponding to negative control (NCA) specific bands of 325 bp, or 425 bp, or 723 bp 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

The all components of the AmpliSens® HPV 16/18-EPh PCR kit should be stored from 2°C to 8°C (except for polymerase Taq (F)) and are stable until the expiry date stated on the label.



Polymerase (Taq F) should be stored at minus 16°C.

## 11. SPECIFICATIONS

### 11.1. Sensitivity

Analytical Sensitivity of AmpliSens® HPV 16/18-EPh PCR kit is no less than  $1 \times 10^3$  genome equivalent in 1 ml of sample (GE/ml).



Claimed analytical features of AmpliSens® HPV 16/118-EPh PCR kit are guaranteed only when additional kits of reagents “DNA-sorb-AM”, or “DNA-sorb-A”, or “DNA-sorb-B”, and “EPh” are used.

### 11.2. Specificity

Specificity of AmpliSens® HPV 16/18-EPh PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

## 12. QUALITY CONTROL

In accordance with Federal State Institution of Science “Central Research Institute of Epidemiology” ISO 13485 –certified Total Quality Management System, each lot of AmpliSens® HPV 16/18-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 *in Vitro* 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