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For Professional Use Only

AmpliSens® *HPV* 6/11-EPh PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® *HPV* 6/11-EPh PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of *Human Papillomavirus* types 6 and 11 DNA in the clinical material by using electrophoretic detection of the amplified products in agarose gel.

The results of PCR analysis are taken into account in complex diagnostics of disease.

2. PRINCIPLE OF PCR DETECTION

Human papillomavirus 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. After PCR the amplified product is detected in agarose gel.

AmpliSens® *HPV* 6/11-EPh PCR kit is based on simultaneous amplification (multiplex-PCR) of *HPV* and β-globin gene DNA parts. The part of human β-globin gene is used as endogenous internal control. PCR analysis for *HPV* types 6 and 11 DNA detection is carried out in one tube. DNA-target selected as endogenous internal control is a part of human genome. It must always present in sample (cervical scrape) in largo manum. This quantity is the same as the cell number in scrape (10^3 – 10^5 genomes per reaction). Hereby the endogenous internal control allows the PCR analysis stages verification (DNA extraction and PCR-amplification) and estimating of sampling and clinical material storage conformity. In case of wrong epithelial scraping (scarcity of epithelial cells) the signal of β-globin gene amplification will be understated. **AmpliSens® *HPV* 6/11-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 ºC. Chemically modified polymerase (TaqF) activates by heating at 95 ºC for 15 min.

3. CONTENT

AmpliSens® *HPV* 6/11-EPh PCR kit is produced in 3 forms:

AmpliSens® *HPV* 6/11-EPh PCR kit variant 100 R (0.5 ml tubes), **REF** V11-100-R0,5-CE.

AmpliSens® *HPV* 6/11-EPh PCR kit variant 100 R (0.2 ml tubes), **REF** V11-100-R0,2-CE.

AmpliSens® *HPV* 6/11-EPh PCR kit variant 50 F, **REF** V11-50F-CE.

AmpliSens® *HPV* 6/11-EPh PCR kit variant 100 R includes:

Reagent	***Description***	***variant 100 R***	
		Volume (ml)	***Quantity***
PCR-mix -1-R *HPV* 6/11 ready-to-use single-dose test tubes (*under wax)*	colorless clear liquid	0.005	110 tubes of 0.5 or 0.2 ml
PCR-mix-2 blue	clear blue liquid	1.2	1 tube
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle
Positive Control DNA *HPV* types 6,11 and human DNA (C+$_{HPV\ 6,11}$)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube

AmpliSens® *HPV* 6/11-EPh PCR kit variant 100 R is intended for 110 reactions, including controls

AmpliSens® *HPV* 6/11-EPh PCR kit variant 50 F includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1 *HPV* 6/11	colorless clear liquid	0.3	1 tube
2.5x PCR-buffer blue	clear blue liquid	0.6	1 tube
Polymerase (TaqF)	colorless clear liquid	0.03	1 tube
Mineral oil for PCR	colorless viscous liquid	2.0	1 tube
Positive Control DNA *HPV* types 6, 11 and human DNA (C+$_{HPV\ 6,11}$)	colorless clear liquid	0.2	1 tube
DNA-buffer	colorless clear liquid	0.5	1 tube

AmpliSens® *HPV* 6/11-EPh PCR kit variant 50 F is intended for 55 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA exraction kit
- Disposable powder-free gloves and laboratory coat
- Detection agarose kit
- 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, Gradient Palm Cycler (Corbett Research, Australia), GeneAmp PCR System 2400, GeneAmp PCR System 2700, (Applied Biosystems), Biometra, MiniCycler, PTC-100 (MJ Research), Terzik (DNA-Technlogy), MaxyGene (Axygen);
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity (for example, Axygen, USA)**.**
- Refrigerator for 2–8 °C.
- Deep-freezer for ≤ –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 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

AmpliSens® *HPV* 6/11-EPh PCR kit is intended for the analysis of DNA extracted with DNA extraction kits from:

- *Cervical or urethral scrapes*

6.1. *Cervical or urethral scrapes*

For women: samples of epithelium are obtained by the same method as used for cytological analysis:

The first method – used kit for sampling consist of one/two cervical cytological brushes and 2 ml volume tube with 0.5 ml of Transport media with mucolytic agent.

Place cervical epithelial swab (endocervix), obtained by the first cervical cytological brush, and/or superficial cervical swab (ectocervix), obtained by the second cervical cytological brush, into the tube with transportation media. The working part of cytological brushes is to be broken off and left in the tube with transport media.

The second method - used kit for sampling, made by Digene (USA), consist of cervical cytological brush and a tube with 1.0 ml of transport media Digene.

Place cervical epithelial swab (endocervix), obtained by cervical cytological brush, into the tube with transportation media Digene.

The third method - used kit for sampling, consist of combined gynecological probe for simultaneous obtaining of epithelium from endo-/exocervix and 5 ml volume tube with 2.0 ml of Transport media with mucolytic agent.

Place cervical epithelial swab (endocervix) and superficial cervical swab (ectocervix) into the tube with transportation media. Working part of the probe is to be broken off and left in the tube with transport media.

The fourth method — used kit for sampling, consist of combined gynecological probe for simultaneous obtaining of epithelium from endo-/exocervix and jar with transport-fixation media made by CytoScreen (Italy) or PreservCyt (USA) for fluid cytology.

Place cervical epithelial swab (endocervix) and superficial cervical swab (exocervix) into the tube with transport-fixation media. Working part of the probe is to be broken off and left in the tube with transport media.

For men: Place the urethral epithelial swab obtained by universal probe, into the 2.0 ml volume tube with 0.5 ml of Transport media with mucolytic agent.

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

7. WORKING CONDITIONS

AmpliSens® *HPV* 6/11-EPh PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. DNA Extraction

It's recommended to use following nucleic acid extraction kit:

- DNA-sorb-AM, **REF** K1-12-100-CE.

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

8.2. Preparing the PCR.

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

8.2.1 Preparing tubes for PCR.

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

1. Collect the required number of tubes prepared as described above or tubes with **PCR-mix-1-R *HPV* 6/11** and wax for amplification of DNA from clinical and control samples.
2. Add **10 µl of PCR-mix-2 blue** to the surface of wax layer, ensuring that it does not fall under the wax and mix with reagents in the tube.
3. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using thermocycler with heating cover this step could be omitted.

AmpliSens® *HPV* 6/11-EPh PCR kit variant 50 F:

1. Prepare the reaction mix for N reactions:

5∗(N+1) µl of PCR-mix-1 *HPV* 6/11

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: two controls and one extra reaction.

It is permitted to mix 2.5x PCR-buffer blue and polymerase (TaqF**)** in advance. Transfer polymerase (TaqF) (0.03 ml) into the tube with 2.5x PCR-buffer blue (0.6 ml). Spin the tube carefully. Mark the tube indicating the date of preparation. Prepared mix is sufficient for 60 reactions. Store 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.

8.2.2 Amplification

Use prepared tubes for PCR. Using tips with aerosol barrier **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction under oil or directly on it.

Carry out the **control amplification reactions:**

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

C+ -Add 10 µl of **Positive Control DNA *HPV* types 6, 11 and human DNA** the tube for Positive Control of Amplification.

Run the following program on the thermocycler (see table 1 or table 2). When the temperature reaches 95ºC (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 s) before their insertion in thermocycler.

Table 1

Programming thermocyclers at DNA amplification of *HPV* types 6, 11 (variant 100 R)

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	GeneAmp PCR System 2400 (Applied Biosystems), Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research)**			**Biometra, MiniCycler, PTC-100 (MJ Research)**		
Step	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 s	42	**95 °C**	15 s	42	**95 °C**	1 min	42
	65 °C	10 s		**65 °C**	25 s		**65 °C**	1 min	
	72 °C	10 s		**72 °C**	25 s		**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 at DNA amplification of *HPV* types 6, 11 (variant 50 F)

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	GeneAmp PCR System 2400 (Applied Biosystems), Terzik (DNA-Technology)			**GeneAmp PCR System 2700 (Applied Biosystems), Gradient Palm Cycler (Corbett Research), MaxyGene (Axygen)**			**Biometra, MiniCycler, PTC-100 (MJ Research)**		
Step	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 s	42	**95 °C**	15 s	42	**95 °C**	1 min	42
	65 °C	10 s		**65 °C**	25 s		**65 °C**	1 min	
	72 °C	10 s		**72 °C**	25 s		**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 carried out 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 the samples are heated to room temperature before running the electrophoresis).

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

- *HPV* type 6 - 260 bp
- *HPV* type 11 - 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

Results interpretation

Table 3

Results for controls

Control	Controlled step	Specific bands in the agarose gel			Interpretation
		260 bp	425 bp	723 bp	
NCA	Amplification	No	No	No	OK
C+	Amplification	Yes	Yes	Yes	OK

- The sample is considered to be positive for *HPV* types 6 and 11 DNA if the bands of 260 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 6 and 11 DNA if the bands of 260 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.

10. 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 suspected.
- If in lane corresponding to a clinical sample the bands of Internal Control (723 bp) is absent it can be suggested that the quantity of epithelial cells insufficient or some mistakes are made during the clinical sampling, DNA extraction or PCR analysis.
- If nonspecific bands at different levels are presented in lines, 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 260 bp, or 425 bp, or 723 bp appeared, then the reagents or samples contamination occurred. In such cases results of analysis are considered to be irrelevant. Test analysis must be repeated and

measures for detecting contamination source must be undertaken.

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

11. TRANSPORTATION

AmpliSens® *HPV* 6/11-EPh PCR kit should be transported at 2–8 ºC for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HPV* 6/11-EPh** PCR kit (except polymerase Taq (F)) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *HPV* 6/11-EPh** PCR kit are to be stable until labeled expiration date. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (Taq F) is to be stored at temperature from minus 24 to minus 16 ºC when not in use.

13. SPECIFICATIONS

13.1. Sensitivity

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

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

13.2. Specificity

Specificity of **AmpliSens® *HPV* 6/11-EPh** PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and clinical trials.

14. QUALITY CONTROL

In accordance with Federal Budget Institute of Science “Central Research Institute for Epidemiology” ISO 13485-Certified Quality Management System, each lot of AmpliSens® *HPV* 6/11-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality.

15. KEY TO SYMBOLS USED

REF	Catalogue number	Σ	Sufficient for
LOT	Batch code		Expiration Date
IVD	*In vitro* diagnostic medical device		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
EC REP	Authorised representative in the European Community	**IC**	Internal control
	Caution		

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
12.11.10	Through the text	Records about PCR kit variant 200 are deleted
25.12.10 KM	Cover page	The phrase “For Professional Use Only” was added
	Intended use	The phrase “The results of PCR analysis are taken into account in complex diagnostics of disease” was added.
	Content	New sections “Working Conditions” and “Transportation” were added
		The “Explanation of Symbols” section was renamed to “Key to Symbols Used”
	Stability and Storage	The information about the shelf life of open reagents was added
	Key to Symbols Used	The explanation of symbols was corrected
21.06.11 VV	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”