CE

IVD For *in Vitro* Diagnostic Use

AmpliSens® HPV 35/45-EPh PCR kit

Instruction Manual

AmpliSens®

EC REP
Ecoli s.r.o., Studenohorska 12
841 03 Bratislava 47
Slovak Republic
Tel.: +421 2 6478 9336
Fax: +421 2 6478 9040
www.ecoli.sk
www.pcrdiagnostics.eu
ecoli@ecoli.sk

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

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1. INTENDED USE.

AmpliSens® HPV 35/45-EPh PCR kit is an in vitro nucleic acid amplification test for qualitative detection and differentiation of *Human papillomavirus* types 35 and 45 DNA in the clinical material by using electrophoretic detection of the amplified products in agarose gel.

2. PRINCIPLE OF PCR DETECTION.

HPV types 35 and 45 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen DNA specific region using special HPV 35/45 primers. After PCR the amplified product is detected in agarose gel. **AmpliSens® HPV 35/45-EPh PCR kit** 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. Wax melting and reaction mix components occur only at 95ºC.

3. CONTENT.

AmpliSens® HPV 35/45-EPh PCR kit is produced in 3 forms:

AmpliSens® HPV 35/45-EPh PCR kit variant 100 R (tubes of 0.5 ml volume), **REF** V14-100-R0,5-CE.

AmpliSens® HPV 35/45-EPh PCR kit variant 100 R (tubes of 0.2 ml volume), **REF** V14-100-R0,2-CE.

AmpliSens® HPV 35/45-EPh PCR kit variant 200, **REF** V14-200-CE.

AmpliSens® HPV 35/45-EPh PCR kit variant 100 R or variant 200 includes:

Reagent	*Description*	*variant 100 R*		*variant 200*	
		Volume (ml)	*Quantity*	*Volume (ml)*	*Quantity*
PCR-mix -1-R HPV 35/45 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-1 HPV 35/45	colorless, clear liquid	---	---	1.1	1 tube
PCR-mix-2 blue	blue clear liquid	1.2	1 tube	1.2	2 tubes
Wax for PCR	white solid	---	---	1.7	2 tubes
Mineral oil for PCR	colorless viscous liquid	4.0	1 dropper bottle	8.0	1 dropper bottle
Positive Control DNA HPV type 35 (C_1+)	colorless, clear liquid	0.2	1 tube	0.2	1 tube
Positive Control DNA HPV type 45 (C_2+)	colorless, clear liquid	0.2	1 tube	0.2	1 tube
DNA-buffer	colorless, clear liquid	0.5	1 tube	0.5	1 tube

AmpliSens® HPV 35/45-EPh PCR kit variant 100 R is intended for 110 reactions, including controls.

AmpliSens® HPV 35/45-EPh PCR kit variant 200 is intended for 220 reactions, including controls.

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES.

- 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
- Thermostatic bath or dry block for tubes with controlled temperature and capability to incubate at temperature between 25 °C and 100 °C.
- Tube racks.
- PCR box
- Personal thermocycler (for example, "Gradient Palm Cycler" (Corbett Research, Australia), "Maxygene" (Axygen, USA) or equivalent)
- 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® HPV 35/45 -EPh PCR kit is intended for analysis of DNA extracted by DNA isolation kits from the clinical material

7. PROTOCOL.

7.1. DNA Isolation

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

- "DNA-sorb-AM", K1-12-100.

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.

When using of AmpliSens® HPV 35/45 -EPh PCR kit variant 100 R steps 1 and 2 are not needed.

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. Add 5 µl of **PCR-mix -1 HPV 35/45** into the bottom of each tube. Add a drop (about 10-15 µl) of melted wax above, ensuring that 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 described above or tubes with **PCR-mix-1-R HPV 35/45** and **wax** for amplification of DNA from clinical and control samples.
4. 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.

5. Add above 1 drop of **mineral oil for PCR** (about 25 µl). When using of thermocycler with heating cover this step could be omitted.

7.2.2 Amplification.

1. Use prepared tubes for PCR. Add **10 µl** of **DNA samples**, obtained from clinical or control samples at the stage of DNA extraction, under or directly above the level of oil by tips with aerosol barrier.
2. Carry out **control amplification reactions:**

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

C_1+ -Add 10 µl of **Positive Control DNA HPV type 35** to the tube for Positive Control of Amplification.

C_2+ -Add 10 µl of **Positive Control DNA HPV type 45** to the tube for Positive Control of Amplification

3. Run the following program on the thermocycler (see table 1). When the temperature will reach 95ºC (pause regimen), insert tubes to cells of amplifier and press button to continue.

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

Table 1

Amplification program of HPV types 35 and 45

	Thermocyclers with active temperature adjustment:						Thermocyclers with block temperature adjustment:		
	"GeneAmp PCR System 2400" (Applied Biosystems)			**"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**	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		**63 °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	

4. Amplification in thermocycler with block temperature adjustment lasts 2 h 30 min, in thermocycler with active temperature adjustment — 1 h 50 min.
5. After the reaction is finished PCR tubes must be collected and sent to the room for PCR products analysis.

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

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

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

- HPV type 35 - 280 bp
- HPV type 45 - 450 bp

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

Results interpretation

Table 2

Results for controls

Control	Which step of test is controlled	Specific bands in the agarose gel 280 bp	Specific bands in the agarose gel 450 bp	Interpretation
NCA	Amplification	No	No	OK
C_1+	Amplification	Yes	No	OK
C_2+	Amplification	No	Yes	OK

- The sample is considered to be positive for HPV types 35 DNA if the bands of 280 bp is present in agarose gel.
- The sample is considered to be positive for HPV types 45 DNA if the bands of 450 bp is present in agarose gel.
- The sample is considered to be negative for HPV types 35 and 45 DNA if the bands of 280 bp and 450 bp are absent.

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 2), then the tests are to be repeated. Remove any reagents that may be suspect.
- If in lane corresponding to positive control (C_1+, C_2+) specific bands of 280 bp and 450 bp (respectively) are not observed, then the result of analysis is irrelevant. It can be caused by mistake in PCR conducting or amplification program fault.
- 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 280 bp or 450 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.

All components of the AmpliSens® HPV 35/45-EPh PCR kit are to be stored at the temperature between 2 ºC and 8 ºC. All components of the PCR kit are to be stable until labeled expiration date.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of AmpliSens® HPV 35/45-EPh PCR kit is no less than 5×10^3 copies per 1 ml of sample (copies/ml).

The claimed analytical features of AmpliSens® HPV 31/33-EPh PCR kit are guaranteed only when additional kits of reagents, "DNA-sorb-AM" and "EPh" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology), are used.

11.2. Specificity.

Specificity of AmpliSens® HPV 35/45-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 31/33-EPh PCR kit is tested against predetermined specifications to ensure consistent product quality

14. EXPLANATION OF SYMBOLS.

	Manufacturer		Temperature limitation
	Use by	LOT	Batch code
IVD	For *in Vitro* Diagnostic Use	VER	Version
REF	Catalogue number	**NCA**	Negative Control of Amplification
	Contains sufficient for <N> tests	EC REP	Authorised representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
C_1+	Positive Control of Amplification	**C_2+**	Positive Control of Amplification