



For *in Vitro* Diagnostic Use

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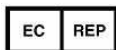
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AmpliSens[®] HPV 16/18-FRT

PCR kit

Instruction Manual

AmpliSens[®]



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1. INTENDED USE.

AmpliSens® HPV 16/18-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of types 16 and 18 of *Human Papillomavirus* (HPV) DNA in the clinical materials (cervical or urethral scrapes) by means of real-time hybridization-fluorescence detection.

2. PRINCIPLE OF PCR DETECTION.

HPV types 16 and 18 detection by the polymerase chain reaction (PCR) is based on the amplification of pathogen genome specific region using special HPV 16/18 primers. In real-time PCR the amplified product is detected using fluorescent dyes. These dyes are linked to oligonucleotide probes which bind specifically to the amplified product. The real-time PCR monitoring of the fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run. **AmpliSens® HPV 16/18-FRT** 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 β -globin gene which is used as the internal endogenous control. Test is running in a single tube.

DNA-target selected as 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 amount of epithelial cells in the specimen is insufficient, amplification signal of β -globin gene will be too low.

AmpliSens® HPV 16/18-FRT PCR kit that allows differentiation of two most carcinogenic virus types is recommended as auxiliary in detection of papillomavirus infection. PCR kits that allow diagnostics of wide range (11-14 types) of highly carcinogenic HPV types such as AmpliSens® HPV HCR screen-Eph (electrophoretic detection in agarose gel), AmpliSens® HPV HCR screen-FRT, AmpliSens® HPV HCR screen titre-FRT (hybridization fluorescence detection) should be applied first.

3. CONTENT.

AmpliSens® HPV 16/18-FRT PCR kit is produced in 1 form:

AmpliSens® HPV 16/18-FRT PCR kit variant screen-titre-FRT (for use with RG, iQ, Mx) **REF** R-V12(RG,iQ,Mx)-CE.

AmpliSens® HPV 16/18-FRT PCR kit variant screen-titre-FRT includes:

<i>Reagent</i>	<i>Description</i>	<i>Volume (ml)</i>	<i>Amount</i>
PCR-mix-1-FEP/FRT HPV 16/18	colorless, clear liquid	0.28	3 tubes
PCR-buffer-FRT	colorless, clear liquid	0.3	3 tubes
Polymerase (TaqF)	colorless, clear liquid	0.02	3 tubes
C1 HPV 16, 18	colorless, clear liquid	0.04	3 tubes
C2 HPV 16, 18	colorless, clear liquid	0.04	3 tubes
C3 HPV 16, 18	colorless, clear liquid	0.04	3 tubes
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 16/18-FRT PCR kit is intended for 108 reactions, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit.
- Disposable powder-free gloves.
- Pipettes (adjustable).
- Sterile pipette tips with aerosol barriers up to 200 μ l.
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2 ml reaction tubes.
- PCR box.
- Rotor-Gene™ 3000 or Rotor-Gene™ 6000 (Corbett Research, Australia) Instrument; iQ5 or iQ iCycler (BioRad, USA) Instrument; Mx3000P or Mx3005P (Stratagene, USA) Instrument.
- Disposable polypropylene microtubes for PCR with 0.5 (0.2) ml capacity (for example, “Axygen”, USA).
- Refrigerator with temperature between 2 and 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 filters 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 and eye protection when handling specimens and reagents. 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 of all specimens and unused reagents in accordance with local regulations.
- Specimens 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 sample or reagent contact with the skin, eyes and mucose membranes. If any of 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 unidirectional manner, beginning in the Extraction Area and moving 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 of biological material samples for PCR-analysis, transportation and storage is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting of the work.

AmpliSens® HPV 16/18-FRT PCR kit is intended for the analysis of 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 using of the following nucleic acid extraction kits:

- "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)

Table 1

7.2. Preparing the PCR.

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

7.2.1 Preparing tubes for PCR.

1. Prepare the mix of PCR-buffer-FRT and polymerase (TaqF) . To do this, transfer the whole amount of the tube with **polymerase (TaqF) (20 µl)** to the tube with **PCR-buffer-FRT (300 µl)**. Vortex carefully to avoid foaming. Indicate the mix preparation time on the tube.



Prepared mix is intended for 40 samples.
Store between 2 and 8 °C for 3 months and use as needed.

2. Prepare the reaction mix (see table 1). Add four control reactions (negative control and three calibrators) and one spare reaction when calculating the reaction mix volume.
3. Each PCR reaction requires:
 - **7 µl of PCR-mix-1-FEP/FRT HPV 16/18**
 - **8 µl of PCR-buffer-FRT and polymerase (TaqF) mix.**



If 40 samples are to be simultaneously analyzed, the simplified way of reaction mix preparation can be applied. The total amount of one tube with PCR-mix-1-FEP/FRT HPV 16/18 and one tube with polymerase (TaqF) should be transferred to the tube with PCR-buffer-FRT.

4. 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.
5. Add **10 µl** of **DNA samples** obtained from clinical or control samples at the stage of DNA extraction into prepared tubes.
6. Carry out control and calibration amplification reactions:

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

HPV calibrators:

- C1** - add **10 µl** of calibrator **C1 HPV 16, 18**, to the tube labeled C1
- C2** - add **10 µl** of calibrator **C2 HPV 16, 18**, to the tube labeled C2
- C3** - add **10 µl** of calibrator **C3 HPV 16, 18**, to the tube labeled C3

Scheme of reaction mix preparation

Number of samples	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
PCR-mix-1-FEP/FRT HPV 16/18, µl	56	63	70	77	84	91	98	105	112	119	126	133	140	147	154
Mix of PCR-buffer-FRT and polymerase (TaqF), µl	64	72	80	88	96	104	112	120	128	136	144	152	160	168	176
Number of samples	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
PCR-mix-1-FEP/FRT HPV 16/18, µl	161	168	175	182	189	196	203	210	217	224	231	238	245	252	259
Mix of PCR-buffer-FRT and polymerase (TaqF), µl	184	192	200	208	216	224	232	240	248	256	264	272	280	288	296

7.2.2. Amplification



AmpliSens-1 RG, AmpliSens-1 iQ, AmpliSens-1 Mx general programs allow simultaneous conducting of any combination of tests (for example, for detection of pathogens of sexually transmitted diseases).

RG

1. Program the Rotor-Gene™ according to manufacturer's manual and Appendix 1.
2. Create a temperature profile on your Rotor-Gene™ instrument as follows:

Amplification program for HPV types 16 and 18 on RotorGene 3000/6000

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	95	15 min	–	1
Cycling	95	15 sec	–	45
	60	35 sec	FAM/Green, JOE/Yellow, ROX/Orange	

AmpliSens-1 RG program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 sec	–	

ROX/Orange and Cy5/Red channels are activated as necessary if they are used in an applied

“multiprime” format test.

3. Make the adjustment of the fluorescence channel sensitivity according to Appendix 1.
4. Define names and types of all samples and insert calibrators’ values as described in Appendix 1 and Important product information bulletin.

iQ

1. Program the iQ according to manufacturer’s manual and Appendix 2.
2. Create a temperature profile on your iQ instrument as follows:

Amplification program for HPV types 16 and 18 on iQ iCycler, iQ5

Step	Temperature, °C	Time	Fluorescence detection	Repeats
1	95	15 min	–	1
2	95	20 sec	–	45
	60	1 min	FAM, HEX, ROX	

AmpliSens-1 iQ program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling 2	95	5 sec	–	40
	60	30 sec	FAM, HEX	
	72	15 sec	–	

ROX and Cy5 channels are activated as necessary if they are used in an applied “multiprime” format test

3. Make the adjustment of the fluorescence channel sensitivity according to Appendix 2.
4. Define names and types of all samples and insert calibrators’ values as described in Appendix 2 and Important product information bulletin.

Mx

1. Program the Mx according to manufacturer’s manual and Appendix 3.
2. Create a temperature profile on your Mx instrument as follows:

Amplification program for HPV types 16 and 18 on Mx3000P, Mx3005P

Step	Temperature, °C	Time	Fluorescence detection	Repeats
1	95	15 min	–	1
2	95	20 sec	–	45
	60	1 min	FAM, HEX/JOE, ROX	

AmpliSens-1 Mx program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Segment 1	95	15 min	–	1
Segment 2 (Cycling)	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Segment 3 (Cycling)	95	5 sec	–	40
	60	30 sec	FAM, JOE, ROX, Cy5	
	72	15 sec	–	

ROX and Cy5 channels are activated as necessary if they are used in an applied “multiprime” format test

3. Make the adjustment of the fluorescence channel sensitivity according to Appendix 3.
4. Define names and types of all samples and insert calibrators’ values as described in Appendix 3 and Important product information bulletin.

8. DATA ANALYSIS.

- HPV type 16 DNA is detected in the FAM/Green fluorescence channel;
- HPV type 18 DNA is detected in ROX/Orange fluorescence channel;
- Endogenous internal control (human DNA) is detected in JOE/HEX/Yellow fluorescence channel.

See **Appendices 1, 2 or 3** for data analysis settings for RG, iQ or Mx Instruments, correspondently.

Results interpretation

The results are interpreted by the software of used device by the crossing (or not) of fluorescence curve with the threshold line.

Results for controls

Control	Stage for control	Ct in channel			Interpretation
		FAM/Green	ROX/Orange	JOE/Yellow/HEX	
C-	DNA isolation	Neg	Neg	Neg	OK
NCA	Amplification	Neg	Neg	Neg	OK
C+	Amplification	Pos	Pos	Pos	OK

Results are accepted as relevant if both positive and negative controls of amplification along with negative control of extraction are passed.

Calibration line plotting and calculating of HPV type 16, HPV type 18, and human DNA copies per PCR sample are automatically performed on the assumption of Ct values and also calibration values that where set (refer to Important product information bulletin). Obtained data are used

for estimation of amount of HPV type 16 or/and 18 DNA per 100,000 cells.

$$\lg \left[\frac{\text{number of copies of HPV (16 or 18) DNA}}{\text{number of copies of human DNA}} \times 2 \cdot 10^5 \right] = \lg(\text{HPV per 100,000 cells})$$

Obtained result is interpreted accordingly to the table below.

lg(HPV per 100,000 cells) data interpretation

lg(HPV per 100,000 cells)	Interpretation
<3	Clinically insignificant.
3-5	Clinically significant. Dysplasia cannot be excluded. Risk of dysplasia development.
>5	Clinically significant, increased. Dysplasia is highly expectable

9. TROUBLESHOOTING.

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

1. If human DNA concentration volume is less than 1,000 copies per tube, it means that either insufficient amount of clinical sample was taken or the errors had occurred during the samples treatment. The sample should be analyzed repeatedly, starting from the DNA extraction or sampling of new clinical material.
2. If the correlation coefficient (R) for calibration line plotting is less than 0.9, all samples should be re-examined starting from the first stage of the analysis.
3. If Ct value appears for negative control of extraction (in the Positive Control channel – FAM/Green, JOE/HEX/Yellow, or ROX/Orange) and for negative control of amplification (in any channel), it suggest of samples or reagents contamination. It is necessary to repeat analysis of all samples and to eliminate the source of contamination.

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens® HPV 16/18-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-1-FEP/FRT HPV 16/18) 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) and PCR-mix-1-FEP/FRT HPV 16/18 are to be stored at not more than minus 16 °C.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® HPV 16/18-FRT** 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 16/18-FRT** 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 16/18-FRT** 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 16/18-FRT** 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 16/18-FRT** 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
	Contains sufficient for <n> tests		Authorised representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000 (Corbett Research)		For working with iQ5, iQiCycler (Bio-Rad)
	For working with Mx3000P or Mx3005P (Stratagene)		