CE

IVD

For Professional Use Only

AmpliSens® *HPV* 16/18-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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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.

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

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 ºC 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:

Reagent	***Description***	***Volume (ml)***	***Amount***
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
DNA calibrator C1 *HPV* 16, 18	colorless clear liquid	0.04	3 tubes
DNA calibrator C2 *HPV* 16, 18	colorless clear liquid	0.04	3 tubes
DNA calibrator 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 µl.
- 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 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 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 are 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

– c*ervical 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-CE.

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-CE.

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-CE.

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

7. WORKING CONDITIONS

AmpliSens® *HPV* 16/18-FRT PCR kit should be used at 18–25 °C.

8. PROTOCOL

8.1. 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)

8.2. Preparing the PCR.

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

8.2.1 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 at 2-8 ºC 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).

DNA calibrators:

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

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

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

Table 1

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

8.2.2. 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 Guidelines [2].
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	***Tepmerature, °C***	***Time***	***Fluorescence detection***	***Repeats***
Hold	95	15 min	–	1
Cycling	95	15 s	–	45
	60	35 s	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 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	20 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 s	–	

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 Guidelines.
4. Define names and types of all samples and insert calibrators’ values as described in Guidelines and *Important product information bulletin*.

iQ

1. Program the iQ according to manufacturer’s manual and Guidelines.
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 s	–	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 s	–	5
	60	20 s	–	
	72	15 s	–	
Cycling 2	95	5 s	–	40
	60	30 s	FAM, HEX, ROX	
	72	15 s	–	

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 Guidelines.
4. Define names and types of all samples and insert calibrators’ values as described in Guidelines and *Important product information bulletin*.

Mx

1. Program the Mx according to manufacturer’s manual and Guidelines.
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 s	–	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 s	–	5
	60	20 s	–	
	72	15 s	–	
Segment 3 (Cycling)	95	5 s	–	40
	60	30 s	FAM, JOE, ROX, Cy5	
	72	15 s	–	

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 Guidelines.
4. Define names and types of all samples and insert calibrators' values as described in Guidelines and *Important product information bulletin*.

9. 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 Guidelines and Important product information bulletin enclosed to PCR kit for data analysis settings for RG, iQ or Mx Instruments.

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.

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

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

10. 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.

11. TRANSPORTATION

AmpliSens® *HPV* 16/18-FRT 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* 16/18-FRT** PCR kit (except for polymerase (TaqF) and PCR-mix-1-FEP/FRT *HPV* 16/18) are to be stored at 2–8 ºC when not in use. They also must be stable until the expiry date stated on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

Polymerase (TaqF) and PCR-mix-1-FEP/FRT *HPV* 16/18 are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

PCR-mix-1-FEP/FRT *HPV* 16/18 is to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® *HPV* 16/18-FRT** PCR kit is no less than $1x10^3$ 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 Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. Specificity.

The analytical 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. The clinical specificity of **AmpliSens ® *HPV* 16/18-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook "Sampling, transportation, storage of clinical material for PCR diagnostics", developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being, Moscow, 2008.
2. Guidelines to AmpliSens® *HPV* 16/18-FRT PCR kit for qualitative detection and differentiation of types 16 and 18 of *human papillomavirus* (*HPV*) DNA by means of real-time hybridization-fluorescence detection developed by Federal Budget Institute of Science "Central Research Institute for Epidemiology".

15. QUALITY CONTROL

In compliance with Federal Budget Institute of Science "Central Research Institute for 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.

16. 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
28.12.10 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	The names of DNA calibrators were given in full (e.g. DNA calibrator C1 *HPV* 16, 18)
		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 reagents before and after the first use was added
		Information that PCR-mix-1-FEP/FRT *HPV* 16/18 is to be kept away from light was added
	Key to Symbols Used	The explanation of symbols was corrected
19.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
20.01.13 LA	Text	Due to replacing appendix 1, 2, 3 with guidelines, references to appendixes were substitute by reference to guidelines