CE

IVD

For Professional Use Only

AmpliSens® *HPV* 6/11-FRT

PCR kit

Instruction Manual

AmpliSens®

EC REP

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1. INTENDED USE

AmpliSens® ***HPV*** **6/11-FRT** PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of human papillomaviruses (*HPV*) types 6 and 11 DNA in the clinical materials (cervical and urethral scrapes) by using real-time hybridization-fluorescence detection.

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

2. PRINCIPLE OF PCR DETECTION

Detection and differentiation of human papillomaviruses (*HPV*) types 6 and 11 DNA is based on simultaneous PCR (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 isolation 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-FRT PCR kit uses “hot-start”, which greatly reduces frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using chemically modified polymerase (TaqF). Chemically modified polymerase (TaqF) activates by heating at 95 ºC for 15 min.

3. CONTENT

AmpliSens® ***HPV*** **6/11-FRT** PCR kit is produced in 1 form:

AmpliSens® *HPV* 6/11-FRT PCR kit variant FRT (for use with RG, iQ, Mx)

REF R-V11(RG,iQ,Mx)-CE.

AmpliSens® ***HPV*** **6/11-FRT** PCR kit, variant FRT includes:

Reagent	***Description***	***Volume (ml)***	***Quantity***
PCR-mix-1-FEP/FRT *HPV* 6/11	colorless clear liquid	0,6	2 tubes
PCR-mix-2-FRT	colorless clear liquid	0,3	2 tubes
Polymerase (TaqF)	colorless clear liquid	0,03	2 tubes
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
Negative Control (C–)*	colorless clear liquid	1.2	1 tube

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

AmpliSens® *HPV* 6/11-FRT PCR kit is intended for 120 reactions, including controls.

4. ADDITIONAL REQUIREMENTS

- DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- 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); iQ5 or iQ iCycler (BioRad, USA); Mx3000P or Mx3005P (Axygen, USA)
- Disposable polypropylene screwing tubes for PCR 1.5 ml volume (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 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 detection.
- 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 one directional 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 samples of biological materials for PCR-analysis, transportation and storage are described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

The following clinical material is used for analysis:

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 with the first cervical cytological brush, and/or superficial cervical swab (ectocervix), obtained with 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 with 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 0.5 ml of Transport media with mucolytic agent.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Isolation

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

- DNA-sorb-AM, **REF** K1-12-100-CE (for clinical material obtained by first, second or third methods).
- DNA-sorb-B, **REF** K1-2-100-CE (for clinical material obtained by first, second or third methods).
- DNA-sorb-C, **REF** K1-6-50-CE (for biopsy samples)

Carry the DNA isolation according to the manufacturer's instructions.

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 required mix of **PCR-mix-2-FRT** and **Polymerase (TaqF).** Transfer the content of the tube with **Polymerase (TaqF)** (30 µl) into the tube with **PCR-mix-2-FRT** (300 µl), mix carefully by vortex mixer. Mark each tube by date of mix preparation.

Prepared mix is intended for 60 reactions and can be stored at 2-8°C for 3 months.

2. Prepare the reaction mix (see Guidelines). During calculation you should take into account the analysis is accompanied by two control steps **(Negative and Positive Controls).** Use for 1 PCR analysis:

- **10 µl** of **PCR-mix-1-FEP/FRT *HPV* 6/11;**
- **5 µ of PCR-mix-2-FRT and Polymerase (TaqF) mix.**

3. Add **15 µl** of prepared reaction mix into needed number of tubes.

4. Using tips with aerosol barrier add **10 µl** of **DNA samples** obtained from clinical or control samples at the DNA extraction stage into prepared tubes.

5. Carry the control amplification reactions:

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

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

8.2.2 Amplification.

8.2.2.1. RG

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

DNA *HPV* types 6 and 11program

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
Hold	95	15 min	–	1
Cycling	95	15 s	–	45
	60	35 s	FAM/Green, JOE/Yellow, ROX/Orange	

This program is single for both kits of reagents **AmpliSens® *HPV* 6/11-FRT** and **AmpliSens® *HPV* 16/18-FRT**.

3. Fluorescence detection is on the 2-nd pass **(60 °C)** in FAM/Green, JOE/Yellow and ROX/Orange fluorometer channels.

The following programs can also be used.

AmpliSens-1 RG program

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle 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	–	

ICy5/Red channel is used if the tests in multiplex format are carried out

DNA *HPV* HCR types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
Hold	95	15 min	–	1
Hold2	65	2 min	–	1
Cycling	95	20 s	–	5
	64 Touchdown: *1 deg. per cycle*	25 s	–	
	65	55 s	–	
Cycling2	95	15 s	–	40
	60	25 s	–	
	65	25 s	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	

The file AmpliSens FRT HR *HPV* Screen RG4x Program.pro attached to AmpliSens® *HPV* HCR screen-titre-FRT can be used for programming.

4. Make the adjustment of the fluorescence channel sensitivity according to Guidelines .

8.2.2.2. iQ

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

DNA *HPV* types 6 and 11program

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
1	95	15 min	–	1
2	95	20 s	–	45
	60	1 min	FAM, HEX, ROX	

This program is single for both kits of reagents **AmpliSens® *HPV* 6/11-FRT** and **AmpliSens® *HPV* 16/18-FRT**.

The following programs can also be used.

AmpliSens-1 iQ program

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
1	95	15 min	–	1
2	95	5 s	–	5
	60	20 s	–	
	72	15 s	–	
3	95	5 s	–	40
	60	30 s	FAM, HEX, ROX, Cy5	
	72	15 s	–	

ICy5/Red channel is used if the tests in multiplex format are carried out

DNA *HPV* HCR types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
Cycle 1	95	15 min	–	1
Cycle 3	95	15 s	–	6
	65 Touchdown: *1 deg. per cycle*	55 s	–	
	65	25 s	–	
Cycle 4	95	15 s	–	41
	60	55 s	FAM/Green, JOE/Yellow, ROX/Orange	
	65	25 s	–	

The file AmpliSens FRT HR *HPV* Screen RG4x Program.pro attached to AmpliSens® *HPV* HCR screen-titre-FRT can be used for programming.

3. Fluorescence detection is on the 60 °C in FAM, JOE and ROX fluorometer channels.
4. Make the adjustment of the fluorescence channel sensitivity according to Guidelines.

8.2.2.2. Mx

3. Program the Mx™ according to manufacturer's manual and Guidelines.
4. Create a temperature profile on your Mx™ instrument as follows:

DNA *HPV* types 6 and 11program

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
1	95	15 min	–	1
2	95	20 s	–	45
	60	1 min	FAM, HEX/JOE, ROX	

This program is single for both kits of reagents **AmpliSens® *HPV* 6/11-FRT** and **AmpliSens® *HPV* 16/18-FRT**.

The following programs can also be used.

AmpliSens-1 Mx program

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle 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, HEX/JOE, ROX, Cy5	
	72	15 s	–	

ICy5/Red channel is used if the tests in multiplex format are carried out

DNA *HPV* HCR types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59

Stage	***Temperature, °C***	***Time***	***Fluorescence detection***	***Cycle repeats***
Segment 1	95	15 min	–	1
Segment 2	65	2 min	–	1
Segment 3 (Cycling)	95	20 s	–	5
	64 Touchdown: *1 deg. per cycle*	25 s	–	
	65	55 s	–	
Segment 4 (Cycling)	95	20 s	–	40
	60	25 s	–	
	65	55 s	Cy5, FAM, HEX/JOE, ROX	

The file AmpliSens FRT HR *HPV* Screen RG4x Program.pro attached to AmpliSens® *HPV* HCR screen-titre-FRT can be used for programming.

9. DATA ANALYSIS

For data processing refer to Guidelines.

The signal is considered to be positive, if the corresponding fluorescence accumulation curve crosses threshold line.

The run result is considered to be **valid** if:

– The signal is absent on all channels (FAM/Green, JOE/Yellow, ROX/Orange) for negative controls ;

– The signals are present on all channels (FAM/Green, JOE/Yellow, ROX/Orange) for positive control.

If the run result is invalid, all obtained data are considered to be invalid, and the reaction must be repeated

The result of *HPV* DNA detection is considered to be:

negative, if IC signal is registered only on ROX/Orange channel and the threshold cycle value doesn't exceed C (see Important Product Information Bulletin).

positive*,* if

- the signal is registered on FAM/Green channel and threshold cycle value doesn't exceed A (positive for *HPV* type 6).
- the signal is registered on JOE/HEX/Yellow channel and threshold cycle value doesn't exceed B (positive for *HPV* type 11).

Absence or threshold cycle acceptable value exceeding is permissible for IC signal (ROX/Orange channel) for given tube if only positive (not doubtful) signal/signals is/are registered on FAM/Green and JOE/HEX/Yellow channel

doubtful, if IC signal is registered, the threshold cycle value doesn't exceed C and

- the signal is registered on FAM/Green channel and threshold cycle value exceeds A, the result is doubtful for *HPV* type 6

- the signal is registered on JOE/HEX/Yellow channel and threshold cycle value exceeds B, the result is doubtful for *HPV* type 11

The doubtful result requires to repeat sample analysis on PCR stage (repeated sampling and/or DNA isolation is not required). If the same or positive result is achieved the DNA *HPV* identification is considered to be positive. If not the DNA *HPV* identification is considered to be negative.

invalid if

- the positive signals are not registered on FAM/Green and JOE/HEX/Yellow channels (*HPV* types 6 and 11) and the IC signal (ROX/Orange) is not registered or the threshold cycle value exceeds C ;

- the doubtful signal/signals is registered on FAM/Green and JOE/HEX/Yellow channels (*HPV* types 6 and 11) and the IC signal (ROX/Orange) is not or the threshold cycle value exceeds C.

The invalid result requires to repeat sample analysis from the beginning DNA isolation or sampling.

10. TROUBLESHOOTING

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

1. If Threshold cycle value is not registered for human DNA (IC) or if it exceeds C the scarcity of clinical material is sampled or an error had place during trial cut. The analysis iteration from the isolation stage and new sampling of clinical material are required.

2. The presence of any Ct value in the results table for negative control of isolation (FAM/Green, JOE/HEX/Yellow or ROX/Orange channels) or PCR (FAM/Green, JOE/HEX/Yellow or ROX/Orange channels) indicates the contamination of samples or reagents. In this case the analysis results are considered to be invalid. The analysis iteration for all samples is required. The measures for identification and elimination of contamination source must be taken.

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

11. TRANSPORTATION

AmpliSens® *HPV* 6/11-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* 6/11-FRT** PCR kit (except for Polymerase (TaqF), PCR-mix-1-FEP/FRT *HPV* 6/11 and PCR-mix-2-FRT) are to be stored at 2–8 ºC when not in use. All components of the **AmpliSens® *HPV* 6/11-FRT** 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 (TaqF), PCR-mix-1-FEP/FRT *HPV* 6/11 and PCR-mix-2-FRT are to be stored at temperature from minus 24 to minus 16 ºC when not in use.

PCR-mix-1-FEP/FRT *HPV* 6/11 is to be stored away from light.

13. SPECIFICATIONS

13.1. Sensitivity.

Analytical Sensitivity of **AmpliSens® *HPV* 6/11-FRT** PCR kit is no less than $1x10^3$ genome equvalents per 1 ml of sample (GE/ml).

The claimed analytical features of **AmpliSens® *HPV* 6/11-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.

Specificity of **AmpliSens® *HPV* 6/11-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* 6/11-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.

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*** **6/11-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

16. KEY TO SYMBOLS USED

REF	Catalogue number		Caution
LOT	Batch code		Sufficient for
IVD	*In vitro* diagnostic medical device		Expiration Date
VER	Version		Consult instructions for use
	Temperature limitation		Keep away from sunlight
	Manufacturer	**NCA**	Negative control of amplification
	Date of manufacture	**C–**	Negative control of extraction
EC REP	Authorised representative in the European Community	**C+**	Positive control of amplification
RG	For working with Rotor-Gene™ 3000/6000 (Corbett Research)	**IC**	Internal control
Mx	For working with Mx3000P or Mx3005P (Axygen, USA)	**iQ**	For working with iQ5, iQ iCycler (Bio-Rad)

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	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* 6/11 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”
18.12.12 LA	3. Content	Short designation of the positive control of amplification was corrected: $C+_{HPV\ 6,11}$ instead of $C+_{6,11}$