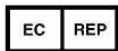




For *in Vitro* Diagnostic Use

AmpliSens® HPV HCR genotype-FRT PCR kit

Instruction manual



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1. INTENDED USE.

AmpliSens® HPV HCR genotype-FRT PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of high carcinogenic risk (HCR) *human papillomaviruses* (HPV) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 DNA in the clinical material (cervical and urethral scrapes) by using real-time hybridization-fluorescence detection of amplified products.

2. PRINCIPLE OF PCR DETECTION.

The test is based on simultaneous PCR (multiplex-PCR) and real-time detection of three HPV types and β -globin gene DNA, used as internal control, in one tube. The analysis of 12 HPV types is carried out in four tubes. Each HPV type is registered on its own channel that allows not only to detect, but also to differentiate the virus genotype. DNA target selected as internal control is a fragment of human genome and must always be presented in sample (cervical swab) in sufficient amount that is equal to the amount of cells in the smear ($10^3 - 10^5$ of genomes). Therefore, endogenous internal control allows not only control stages of PCR (DNA isolation and PCR performance) but also evaluation of material obtaining and storage adequacy. If epithelial swab is obtained with mistakes (number of epithelial cells is insufficient), amplification signal of β -globin gene will be lowered.

3. CONTENT.

AmpliSens® HPV HCR genotype-FRT PCR kit is produced in 1 form:

AmpliSens® HPV HCR genotype-FRT PCR kit variant FRT REF R-V25 (RG,iQ,Mx)-CE.

AmpliSens® HPV HCR genotype-FRT PCR kit variant FRT includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FRT HPV16/18/31	colorless, clear liquid	0.08	6 blue cap tubes
PCR-mix-1-FRT HPV 39/45/59	colorless, clear liquid	0.08	6 pink cap tubes
PCR-mix-1-FRT HPV 33/35/56	colorless, clear liquid	0.08	6 green cap tubes
PCR-mix-1-FRT HPV 51/52/58	colorless, clear liquid	0.08	6 orange cap tubes
PCR-buffer-FRT	colorless, clear liquid	1.1	2 tubes
Polymerase (TaqF)	colorless, clear liquid	0.06	2 tubes
Positive Control DNA HPV types 16, 18, 31 and human DNA	colorless, clear liquid	0.06	1 tube
Positive Control DNA HPV types 39, 45, 59 and human DNA	colorless, clear liquid	0.06	1 tube
Positive Control DNA HPV types 33, 35, 56 and human DNA	colorless, clear liquid	0.06	1 tube
Positive Control DNA HPV types 51, 52, 58 and human DNA	colorless, clear liquid	0.06	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 isolation procedure as Negative control of Extraction

AmpliSens® HPV HCR genotype-FRT PCR kit is intended for 108 tests, including controls.

4. ADDITIONAL REQUIREMENTS.

- DNA isolation kit
- Disposable powder-free gloves and laboratory coat
- Pipettes (adjustable)
- Sterile DNase-free 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 (BioRad, USA) Instrument; Mx3000P (Stratagene, USA) Instrument.
- Disposable polypropylene microtubes for PCR with 0.5 ml (0.2) capacity
- Refrigerator for 2–8°C
- Deep-freezer with temperature not more than –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 is described in manufacturer's handbook [1]. It is recommended that this handbook is read before starting work.

Clinical material:

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. PROTOCOL.

7.1. DNA Isolation

It's recommended to use the following nucleic acid extraction kits (see table 1):

Table 1

Material	DNA isolation kit	REF
Clinical material obtained by the 1 st , 2 nd , or 3 rd method	DNA-sorb-B	K1-2-100-CE
	DNA-sorb-AM	K1-11-100-CE
Biopsy material from mucosa	DNA-sorb-C	K1-6-50-CE



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

Note, that nor positive control, nor internal control are used in isolation procedure.

7.2. Preparing the PCR.

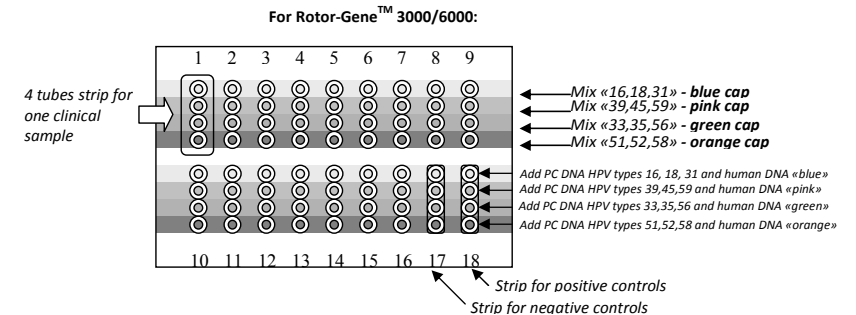
Total reaction volume is **13 µl**, the volume of DNA sample is **5 µl**.

1. Prepare required number of tubes (0,1ml) (for 0,2 ml tubes with Rotor-Gene™ 6000 add 1 drop of mineral Oil to each tube).
2. Add the whole volume of **Polymerase (TaqF)** (60 µl) into the tube with **PCR-buffer-FRT** (1100 µl). Carefully vortex the tube.
This mix is intended for 54 samples, including controls, and is stable for 3 months at +4°C.
3. Add **90 µl of Polymerase (TaqF)** and **PCR-buffer-FRT** mix into each of the four **PCR-mix-1** tubes and carefully vortex the tubes (sufficient for genotyping of 16 clinical samples, including control samples reactions).
4. If it is necessary to test less than 16 samples prepare for each PCR-mix-1 one new tube and add for each sample **3,5*(N+2) µl of PCR-mix-1, 4,5*(N+2) of Polymerase (TaqF) and PCR-buffer-FRT mix**.

For example, for 8 clinical samples and 2 controls (Positive and Negative) prepare 35 µl of every PCR-mix-1 (3,5*[8+2]) and add 45 µl of Polymerase (TaqF) and PCR-buffer-FRT mix.

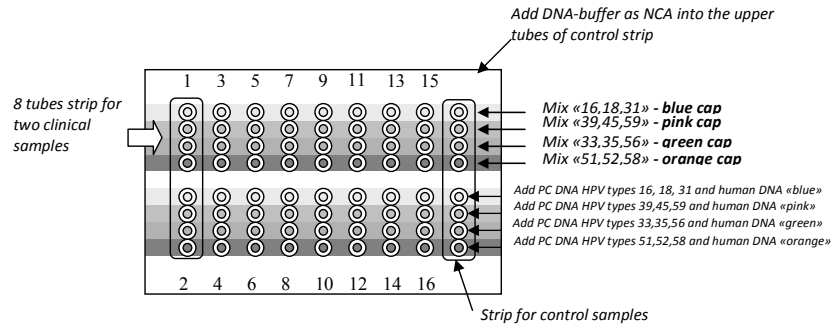
The reaction mix (containing PCR-mix-1, Polymerase (TaqF) and PCR-buffer-FRT) should be used within 2 hours.

5. Add **8,0 µl of Reaction Mix** into each tube. Dispense Reagents and Samples as shown below (every sample has to be tested in 4 tubes): Add to the first strip tube 8,0 µl of mix "16,18,31" (blue cap), to the second strip tube 8,0 µl of mix "39,45,59" (pink cap), to the third strip tube 8,0 µl of mix "33,35,56" (green cap), to the fourth tube 8,0 µl of mix "51,52,58" (orange cap).
When using 8-tubes strip, the tubes 5,6,7,8 are prepared in the same way.



Place strips as shown on the picture above. For 16 samples 18 strips are needed (for N samples – N+2 strips are needed).

For iQ5™ and Mx3000P™:



Place strips as shown on the picture above. For 16 samples 9 strips are needed (for N samples – N/2+1 strips are needed).



Be careful not to change the reaction mixes order in strips.

Add 5 µl of extracted DNA sample into 4 tubes with different reaction mixes.

6. Perform control amplification reactions:

NCA Add 5 µl of DNA-buffer into 4 tubes with different reaction mixes.

add 5 µl of Positive Control DNA HPV types 16, 18, 31 and human DNA into the tube with “16,18,31” reaction mix;

C+ add 5 µl of Positive Control DNA HPV types 39, 45, 59 and human DNA into the tube with “39,45,59” reaction mix;

add 5 µl of Positive Control DNA HPV types 33, 35, 56 and human DNA into the tube with “33,35,56” reaction mix;

add 5 µl of Positive Control DNA HPV types 51, 52, 58 and human DNA into the tube with “51,52,58” reaction mix;

7.3. Amplification

Program the Real-time instrument according to manufacturer’s manual and Appendix 1.

7.3.1. For Rotor-Gene™ run one of the following amplification programs (see tables 2 and 3).

Table 2

AmpliSens-1 RG amplification program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	95	15 min	–	1
Cycling	95	15 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
Cycling2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
	72	15 sec	–	

Table 3

RG amplification program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Hold	95	15 min	–	1
Cycling	95	15 sec	–	45
	60	30 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	

Set parameters of calibration in the wizard of new experiment:

- Calibrate on FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red channels;
- Calibrate before the 1st measurement;
- Set channel calibration for all dyes: from 4FI to 8FI;

7.3.2. For iQ iCycler™, iQ™ run one of the following amplification programs (see tables 4 and 5).

Table 4

AmpliSens-1 iQ amplification program

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

Table 5

iQ amplification program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Cycle 1	95	15 min	–	1
	95	15 sec	–	
Cycle 2	60	50 sec	FAM, JOE/HEX, ROX, Cy5	45
	72	15 sec	–	

7.3.3 For Mx3000P™ and Mx3005P™ run one of the following amplification programs (see tables 6 and 7).

Table 6

AmpliSens-1 Mx amplification 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	–	

Table 7

Mx amplification program

Step	Temperature, °C	Time	Fluorescence detection	Repeats
Segment 1	95	15 min	–	1
Segment 2	95	20 sec	–	45
	60	60 sec	Cy5, FAM, HEX, ROX	

8. DATA ANALYSIS.

For data analysis refer to Appendix 1 (for Rotor-Gene™), to Appendix 2 (for iQ iCycler™), to Appendix 3 (for Mx3000P™).

Signal in the tube on the channel is considered to be positive, if the corresponding fluorescence accumulation curve cross threshold line. The signal is characterized by threshold cycle — that is, the cycle corresponding to the cross point of fluorescence curve and threshold line.

The result is **valid** if:

- Negative controls contain no signal on all channels (FAM/Green, JOE/Yellow/HEX/TET, ROX/Orange, Cy5/Red);
- All 12 HPV HCR types are detected in positive control samples.



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

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

- **invalid**, if no positive signal is detected on any channel in any strip tube, including IC channel (Cy5/Red). (For Rotor-Gene™ only: sample is considered to be invalid, if only IC signal is detected, but Ct value exceeds 30 (or 35, if running Amplisens-1 program)).
- **negative**, if IC signal is present in all four tubes (Cy5/Red channel) and positive signals are not detected on any other channel

(FAM/Green, JOE/Yellow, ROX/Orange).

- *positive*, in all other cases. IC signal can be absent in positive samples.



For Rotor-Gene™ only: the sample is considered to be *weak*, if IC signal (Cy5/Red channel) is present in all strip tubes and Ct value is **less than 30** (or **35**, if running Amplisens-1 program) and there is a positive signal on any other channel that **exceed 35** (*questionable* result for this HPV type).

For this sample PCR run has to be repeated. If in the second run the result is *positive*, the sample is considered to be *positive*. If in the second run the result is *weak* or *negative*, the sample is considered to be *negative*.

9. TROUBLESHOOTING.

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

1. If for Negative Controls (C-, NCA) any Ct value appears on any channel, it indicates the contamination of the reagents or samples. In this case results of the analysis for all samples are considered to be invalid. It is necessary to repeat the analysis of all tests, and to take measures to detect and eliminate the source of contamination.
2. If in positive control samples not all 12 HPV HCR types are detected, it can suggest incorrect programming of the temperature profile of the instrument, incorrect configuration of the PCR reaction, or storage conditions of the kit components has not complied with the manufacturer's instruction, or the reagent kit has expired. Accurate programming of the instrument (see 7.2.2.2.), storage conditions, and the expiration date of the reagents should be checked, and then the PCR should be repeated.

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

10. STABILITY AND STORAGE.

The all components of the **AmpliSens® HPV HCR genotype-FRT PCR kit** (except for PCR-mix-1-FRT HPV16/18/31, PCR-mix-1-FRT HPV 39/45/59, PCR-mix-1-FRT HPV 33/35/56, PCR-mix-1-FRT HPV 51/52/58, Polymerase (TaqF)) should be stored between 2 and 8 °C. The all components of the **AmpliSens® HPV HCR genotype-FRT PCR kit** are to be stable until the expiry date stated on the label.



PCR-mix-1-FRT HPV16/18/31, PCR-mix-1-FRT HPV 39/45/59, PCR-mix-1-FRT HPV 33/35/56, PCR-mix-1-FRT HPV 51/52/58 and Polymerase (TaqF) are to be stored at minus 16 °C.

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical sensitivity of **AmpliSens® HPV HCR genotype-FRT PCR kit** is no less than 1×10^3 genome equivalents per 1 ml of sample for 16,18,31,33,35,39,45,51,52,56,58 and 59 types.



The claimed analytical features of **AmpliSens® HPV HCR genotype-FRT PCR kit** are guaranteed only when additional reagents kits "DNA-sorb-B", "DNA-sorb-AM", "DNA-sorb-C", "DNA-sorb-D" (manufactured by Federal State Institution of Science Central Research Institute of Epidemiology) are used.

11.2. Specificity.

Specificity of **AmpliSens® HPV HCR genotype-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 HCR genotype-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 HCR genotype-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 complex
	Contains sufficient for <n> tests		Authorized representative in the European Community.
	Consult instructions for use		Caution, consult accompanying documents
	For working with Rotor-Gene™ 3000/6000		For working with IQ5, IQ iCycler
	Positive control		Negative control