



For *in Vitro* Diagnostic Use

AmpliSens® HPV HCR screen-titre-FRT

PCR kit

Instruction Manual



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1. INTENDED USE.

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

AmpliSens® HPV HCR screen-titre-FRT PCR kit is capable to find (without genotype detecting) the HPV DNA of two main phylogenetic groups – A7, A9, which include the following 10 types: 16, 18, 31, 33, 35, 39, 45, 52, 58, 59 – as well as the HPV DNA 51 (A5 group) and 56 (A6 group) types. These types have in possession the high transforming activity. They are also responsible for more over 94 per cent of cervical dysplasia and *cervix uteri* cancer.

AmpliSens® HPV HCR screen-titre-FRT PCR kit PCR kit is adapted for two channel devices, for example «Rotor-Gene» 3000/6000 («Corbett Research», Australia), «iQ iCycler» («BioRad», USA) with filters for FAM and HEX/JOE channel, «SmartCyclerII» («Cepheid», USA), or for four channel devices, for example «Rotor-Gene» 3000/6000 («Corbett Research», Australia), «Mx3000P» («Stratagene», USA), «iQ5» («BioRad», USA).

2. PRINCIPLE OF PCR DETECTION.

The method is based on simultaneous real-time amplification (multiplex PCR) and real-time detection of E1-E2 HPV genes DNA fragments and a fragment of β-globin gene DNA which is used as internal endogenous control. PCR analysis for HPV 12 types DNA presence is carried out in two tubes (PCR kit variant screen-titre-FRT 2x) or in a single tube (PCR kit variant screen-titre-FRT 4x).

The result of HPV DNA amplification is registered on JOE/Yellow/HEX/TET fluorescent channel in case of two channel device usage. Detection of genotypes belonging to A9 phylogenetic group (16, 31, 33, 35, 52, 58) is registered in one tube, detection of genotypes from phylogenetic group A7 (18, 39, 45, 59), 51 and 56 genotypes are registered in the other tube.

In case of four channel device usage the result of amplification of each phylogenetic group HPV DNA is registered on separate fluorescent channel (A9 – JOE/Yellow, A7 – ROX/Orange, 51 and 56 types – on channel Cy5/Red).



Detection of phylogenetic groups in different tubes is not considered to be a virus genotyping because each group consists of different HPV-genotypes.

Result of Internal Control amplification is registered on FAM/Green channel. DNA-target selected as endogenous internal control is the fragment of human genome. It must be always present in a sample (cervical scrape) in adequate quantity what equivalent to the quantity of cells in the swab (10^3 - 10^5 genomes). 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.

Quantitative analysis of HPV DNA is based on linear dependence between the cycle of sample fluorescent signal increasing (Cycle threshold, Ct) and initial concentration of HPV DNA. DNA-calibrators – samples with determined concentration of HPV DNA are simultaneously amplified for assay. The concentration of HPV DNA in samples is defined according to the calibration line constructed on results of DNA-calibrators amplification. AmpliSens® HPV HCR screen-titre-FRT PCR kit PCR kit also uses the principle of quantitative result normalization. It is a correlation of obtained HPV DNA concentration with genome DNA in order to grade the effect of variation in sampling.

AmpliSens® HPV HCR screen-titre-FRT 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 or chemically modified polymerase (TaqF). The wax melting and reaction mix component occurs only at 95 °C. Chemically modified polymerase (TaqF) activates by heating at 95 °C

3. CONTENT.

AmpliSens® HPV HCR screen-titre-FRT PCR kit is produced in 2 forms:

AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 2x, **REF**R-V31-T-2x-CE (RG,iQ,SC) (for use with RG, iQ, SC).

AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 4x, **REF**R-V31-T-4x-CE (RG,iQ,Mx) (for use with RG, iQ, Mx).

AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 2x includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix-1-FRT HPV A9	colorless, clear liquid	0.14	6 blue cap tubes
PCR-mix-1-FRT HPV A7+	colorless, clear liquid	0.14	6 green cap tubes
PCR-buffer-FRT	colorless, clear liquid	0.30	6 tubes
Polymerase (TaqF)	colorless, clear liquid	0.02	6 tubes
K1 HPV 16, 18	colorless, clear liquid	0.04	3 tubes
K2 HPV 16, 18	colorless, clear liquid	0.04	3 tubes
K3 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

AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 2x is intended for 216 reactions (108 tests), including controls.

AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 4x includes:

Reagent	Description	Volume (ml)	Quantity
PCR-mix -1- FRT HPV screen-titre	colorless, clear liquid	0.28	3 tubes
PCR-buffer-FRT	colorless, clear liquid	0.30	3 tubes
Polymerase (TaqF)	colorless, clear liquid	0.02	3 tubes
K1 HPV	colorless, clear liquid	0.04	3 tubes
K2 HPV	colorless, clear liquid	0.04	3 tubes
K3 HPV	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

AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 4x is intended for 108 reactions, including controls.

PCR kit also contains the following:

1. Compact Disk includes:

- software (Microsoft® Excel format) for data interpretation and result analysis obtaining;
- template file in "Rotor-Gene" software format for fast run of experiment;
- template file in "Mx3000P" software format for fast run of experiment;
- amplification program file for "Rotor-Gene" and "iQ iCycler" software;

2. Instruction manual (paper document).

3. Appendixes, which contain detailed programming and data processing description.

4. Bulletin, which contain calibration values for determinate lot of AmpliSens® HPV HCR screen-titre-FRT PCR kit.

4. ADDITIONALLY REQUIRED MATERIALS, REAGENTS AND DEVICES.

- 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
- Refrigerator for 2–8 °C
- Deep-freezer with temperature not more than minus16°C.
- Reservoir for disposed tips.
- Two-channel real-time genetic amplification detection system: "Rotor-Gene" 3000/6000 (Corbett Research, Australia), "iQ iCycler" (Bio-Rad, USA) with filters for channels FAM and HEX/JOE filters; "SmartCyclerII" (Cepheid, USA) or equivalent for AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 2x.
- Four-channel real-time genetic amplification detection system: "Rotor-Gene" 3000/6000 (Corbett Research, Australia), "Mx3000P" (Stratagene, USA), "iQ5" (BioRad, USA) or equivalent for AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 4x
- "Rotor-Gene": disposable polypropylene undomed and unstriped 0.2 ml microtubes for PCR (for instance, "Axygen", USA) for 36-well rotor or 0.1 ml microtubes (Corbett Research, Australia) for 72-well rotor.
- "iQ5", "iQ iCycler": disposable polypropylene domed 0.2 ml microtubes for PCR (for instance, "Axygen", USA), stripe domed tubes or 96-wells plate for PCR equipped with heat-sealing optical transparent films (Bio-Rad, USA).
- "Mx3000P": disposable polypropylene domed and stripe/unstriped 0.2 ml microtubes for PCR (for example, "Axygen", USA) for 36-well rotor or plate for PCR equipped with heat-sealing optical transparent films (Bio-Rad, USA).
- "SmartCyclerII": disposable polypropylene microtubes for PCR adjustable for "SmartCyclerII" and intended for 25 µl of reaction mix (Cepheid, USA). "MiniSpin" centrifuge and tube rack (Cepheid, USA).

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 protective gloves, laboratory coats protect eye 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 biological cabinet in accordance with appropriate biosafety practices.
- Clean and disinfect all spills of specimens or reagents 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.

AmpliSens® HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT is intended for the analysis of DNA extracted with DNA isolation kits from:

- Cervical or urethral scrapes

6.1. Cervical or urethral scrapes

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 of transport media "TSM" [REF](#) 953.

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 of transport media "TSM" [REF](#) 953.

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 (ectocervix) 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 of transport media

"TSM" [REF](#) 953.



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

7. PROTOCOL.

7.1. 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).



Please carry out the DNA isolation according to the manufacturer's instruction.



Note, that neither Positive Control, nor Internal Control is used in isolation procedure.

7.2. Preparing the PCR.

Total reaction volume - 25 µl, volume of DNA sample - 10 µl.

7.2.1. Preparing tubes for PCR using HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 2x.

1. Preparation of reaction mix for desired number of samples (tables 2 and 3). During calculations it should be noted that every run needs at least four control points (negative control and three calibrators). Besides, it is necessary to take more quantity of reagents than needed to calculate reaction mix for one more extra reaction. Each amplification reaction requires:

- **7.0 µl of PCR-mix-1-FRT HPV A9 or PCR-mix-1-FRT HPV A7+;**
- **7.5 µl of PCR-buffer-FRT;**
- **0.5 µl of polymerase (TaqF).**

Table 2

Methods of transferring the reagents into the tubes

If 14 samples plus controls are to be analyzed	If less than 14 samples (for example, N) plus 8 controls are to be analyzed
<ul style="list-style-type: none"> • Collect one tube of each of the following reagents: polymerase (TaqF), PCR-buffer-FRT, PCR-mix-1-FRT HPV A9 and PCR-mix-1-FRT HPV A7+. • Transfer the whole volume (20 µl) of polymerase (TaqF) into the tube with PCR-buffer-FRT (300 µl). Carefully vortex the tube (minimum speed), then centrifuge on vortex for 1 sec. Avoid foaming. • Add 160 µl of prepared mix into each of tubes with PCR-mix-1-FRT HPV A9 (140 µl) and PCR-mix-1-FRT A7+ (140 µl) 	<ul style="list-style-type: none"> • In a separate tube mix 15*(N+5) µl of PCR-buffer-FRT and 1.0*(N+5) µl of polymerase (TaqF). Carefully vortex the tube at minimum speed, then centrifuge on vortex for 1 sec. • Into two separate tubes add 7 *(N+5) of PCR-mix-1-FRT HPV A9 and PCR-mix-1-FRT HPV A7+. • The half (8*(N+5) µl) of prepared mix of PCR-buffer-FRT with polymerase (TaqF) transfer into the tube that contains PCR-mix-1-FRT HPV A9; the other half (8*(N+5) µl) of prepared mix transfer into the tube with PCR-mix-1-FRT HPV A7+. (Refer to table 2 for calculation tips).
- Carefully vortex prepared mixes (minimum speed), then centrifuge on vortex for 1 sec. Avoid foaming.	
- Prepare 36 PCR tubes.	- Prepare (N*2 +8) PCR tubes (2 tubes per each clinical samples plus 8 tubes for controls).



Mix of polymerase (TaqF) and PCR-buffer-FRT are to be stored at temperature between 2°C and 8°C for 3 month.
Mix of polymerase (TaqF), PCR-buffer-FRT, and PCR-mix-1-FRT HPV A9 as well as mix of polymerase (TaqF), PCR-buffer-FRT, and PCR-mix-1-FRT HPV A7+ should be used within 2 hours after preparation.

Table 3

Scheme of reaction mix preparation for n analyzed samples, negative control and three calibrators.

- Mix in a separate tube.

Number of samples, N	1	2	3	4	5	6	7
PCR-buffer-FRT, µl	90	105	120	135	150	165	180
Polymerase (TaqF), µl	6	7	8	9	10	11	12
Number of samples, N	8	9	10	11	12	13	14
PCR-buffer-FRT, µl	195	210	225	240	255	270	Whole tube
Polymerase (TaqF), µl	13	14	15	16	17	18	Whole tube

- Mix in a separate tube the part of prepared mix of PCR-buffer-FRT and polymerase (TaqF) with PCR-mix-1-FRT HPV A9 (blue cup).

Number of samples, N	1	2	3	4	5	6	7
mix of PCR-buffer-FRT and polymerase (TaqF), µl	48	56	64	72	80	88	96
PCR-mix-1-FRT A9, µl (blue cup)	42	49	56	63	70	77	84
Number of samples, N	8	9	10	11	12	13	14
mix of PCR-buffer-FRT and polymerase (TaqF), µl	104	112	120	128	136	142	150
PCR-mix-1-FRT A9, µl (blue cup)	91	98	105	112	119	126	Whole tube

In a separate tube transfer the part of prepared mix of PCR-mix-FRT and polymerase (TaqF) and add PCR-mix-1-FRT HPV A7+ (green cup).

Number of samples, N	1	2	3	4	5	6	7
mix of PCR-buffer-FRT and polymerase (TaqF), µl	48	56	64	72	80	88	96
PCR-mix-1-FRT A7+, µl (green cup)	42	49	56	63	70	77	84
Number of samples, N	8	9	10	11	12	13	14
mix of PCR-buffer-FRT and polymerase (TaqF), µl	104	112	120	128	136	142	150
PCR-mix-1-FRT A7+, µl (green cup)	91	98	105	112	119	126	Whole tube

2. Into the half of the tubes add **15 µl** of prepared mix of HPV FRT A9 (per each tube) into the other half add **15 µl** prepared mix of HPV FRT A7+ (per each tube).
3. Into prepared tubes for PCR using tips with aerosol barrier add **10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction. First add DNA samples into the tubes with prepared mix of HPV FRT A9 and then into the tubes with HPV FRT A7+ prepared mix (scheme 1).



Ensure that the sorbent is not transferred in the PCR reaction mix while adding DNA samples.

4. Carry out the control amplification reactions:

NCA

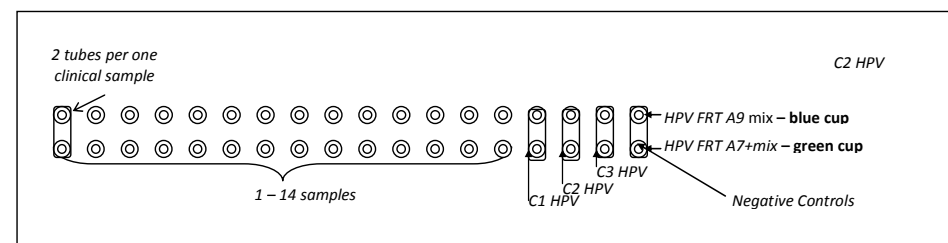
Add **10 µl of DNA-buffer** instead of DNA sample into the tube with HPV FRT A9 prepared mix and into the tube with HPV FRT A7+

HPV

calibrators (K1, K2, K3) Into each of three tubes with HPV FRT A9 prepared mix add 10 µl of each of HPV DNA calibrators (**K1 HPV 16, 18, K2 HPV 16, 18, K3 HPV 16, 18**); into each of three tubes with HPV FRT A7+ prepared mix add **10 µl** of each of HPV DNA calibrators (**K1 HPV 16, 18, K2 HPV 16, 18, K3 HPV 16, 18**).

Scheme 1

Tubes order and reagent insertion (only if the tubes are to be used)¹.



7.2.2. Amplification.

1. Place the tubes in the reaction chamber.

For "Rotor-Gene"™ run one of the two following programs. For detailed programming refer to Appendix 1.

Table 4

Amplification program for HCR HPV 16, 18, 31, 33, 35; 39, 45, 51, 52, 56, 58, 59 types DNA

Step	Temperature, °C	Time	Fluor. measurement	No of repeats
Hold	95	15 min	-	1
Hold2	65	2 min	-	1
Cycling	95	20 sec	-	5
	64	25 sec	-	
	Touchdown: 1 deg. per cycle / Lower temperature of each step on 1°C			
Cycling2	65	55 sec	-	40
	95	15 sec	-	
	60	25 sec	-	
	65	40 sec	FAM/Green, JOE/Yellow	

¹ In case the PCR plate is used ("iQ iCycler") it is necessary to insert samples according to the tube setting order (refer to the Appendix 2).



Also **universal program** for amplification and detection “**AmpliSens-1 RG**” can be used (see Table 5). This program allows any combinations of tests simultaneously on one device and with unified program (for example, combined with tests for STD pathogens DNA detection).

Analytic features of the reagent set while using the universal amplification program are the same.

Table 5

Amplification program “AmpliSens-1 RG”				
Step	Temperature, °C	Time	Fluor. measurement	No of repeats
Hold	95	15 min	–	1
	95	5 sec	–	5
Cycling	60	20 sec	–	
	72	15 sec	–	
Cycling2	95	5 sec	–	40
	60	20 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	
			72	

For “iQ iCycler”™ run one of the two following programs. For detailed programming refer to Appendix 2.

Table 6

Amplification program for HCR HPV 16, 18, 31, 33, 35; 39, 45, 51, 52, 56, 58, 59 types DNA				
Step	Temp	Time	Fluor. measurement	No of repeats
1	95 °C	15 min	–	1
2	65 °C	2 min	–	1
	95 °C	20 sec	–	
3	64 °C	25 sec	–	5
	Temp -; 1.0 for a cycle			
	65 °C			
4	95 °C	55 sec	–	42
	95 °C	20 sec	–	
	60 °C	25 sec	–	
	65 °C	55 sec	FAM, HEX	



Also **universal program** for amplification and detection “**AmpliSens-1 iQ**” can be used (see Table 8). This program allows any combinations of tests simultaneously on one device and with unified program (for example, combined with tests for STD pathogens DNA detection).

Analytic features of the reagent set while using the universal amplification program are the same.

Table 7

Amplification program “AmpliSens-1 iQ”				
Step	Temp	Time	Fluor. measurement	No of repeats
1	95 °C	15 min	–	1
2	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
3	95 °C	5 sec	–	40
	60 °C	30 sec	FAM, HEX, ROX, Cy5	
	72 °C	15 sec	–	

For “SmartCycler”™ run the following programs. For detailed programming refer to Appendix 3.

Table 8

Amplification program for HCR HPV 16, 18, 31, 33, 35; 39, 45, 51, 52, 56, 58, 59 types DNA				
Step	Temperature, °C	Time	Fluor. measurement	No of repeats
Stage 1. Hold	95	900 sec	–	1
Stage 2. Hold	65	120 sec	–	1
Stage 3. 3-Temperature Cycle	95	20 sec	–	5
	63	30 sec	–	
	65	60 sec	–	

Stage 4. 3-Temperature Cycle	95	25 sec	–	42
	60	30 sec	–	
	65	60 sec	Switched on	

7.2.3. Preparing tubes for PCR using HPV HCR screen-titre-FRT PCR kit variant screen-titre-FRT 4x.

1. First it needs to prepare the mix of **PCR-buffer-FRT** and **polymerase (TaqF)**. The content of one tube with **polymerase (TaqF) (0.02 ml)** transfer into the tube with **PCR-buffer FRT (0.3 ml)**. Carefully vortex the tube. Avoid foaming. Mark each tube with mix preparation date. For reagent mix use only sterile tips with aerosol barriers.



Prepared mix is intended for 40 reactions. The mix is to be stored at temperature between 2 and 8 °C during 3 months. It can be used when need.

2. Transfer the reagents into tubes according to table 9.

Table 9

Methods of transferring the reagents into the tubes

1 st method	2 nd method
<ol style="list-style-type: none"> Add 7 µl of PCR-mix-1-FRT HPV screen-titre into tubes Add 8 µl of prepared mix of PCR-buffer-FRT and polymerase (TaqF) 	<ol style="list-style-type: none"> Prepare the reaction mix for needed number of reaction – mix in a separate tube PCR-mix-1-FRT HPV screen-titre and prepared mix of PCR-buffer FRT and polymerase (TaqF). The following quantity of reagents is needed for each reaction: <ul style="list-style-type: none"> 7 µl of PCR-mix-1-FRT HPV screen-titre 8 µl of PCR-buffer-FRT and polymerase (TaqF) mix During calculations it should be noted that every run needs at least four control points (negative control and three calibrators). Besides, it is necessary to take more quantity of reagents than needed to calculate reaction mix for one more extra reaction (see table 10). Add 15 µl of prepared mix into tubes



Mix of polymerase (TaqF), PCR-buffer-FRT, and PCR-mix-1-FRT HPV A9 as well as mix of polymerase (TaqF), PCR-buffer-FRT, and PCR-mix-1-FRT HPV A7+ should be used within 2 hours after preparation.

Table 10

Scheme of reaction mix preparation for n analyzed samples, negative control and three calibrators.

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

3. Into prepared tubes for PCR using tips with aerosol barrier **add 10 µl of DNA samples**, obtained from clinical or control samples at the stage of DNA extraction.



Ensure that the sorbent is not transferred in the PCR reaction mix while adding DNA samples.

4. Carry out the control amplification reactions:

NCA	Add 10 µl of DNA-buffer
HPV calibrators (K1, K2, K3)	Add into three 10 µl of each of HPV DNA calibrators (K1 HPV, K2 HPV, K3 HPV)

7.2.4. Amplification.

1. Place the tubes in the reaction chamber.

For "Rotor-Gene" TM run one of the two following programs. For detailed programming refer to Appendix 4.

Table 11

Amplification program for HCR HPV 16, 18, 31, 33, 35; 39, 45, 51, 52, 56, 58, 59 types DNA				
Step	Temperature, °C	Time	Fluor. measurement	No of repeats
Hold	95	15 min	–	1
Hold2	65	2 min	–	1
Cycling	95	20 sec	–	5
	64	25 sec	–	
	Touchdown: 1 deg. per cycle / Lower temperature of each step on 1°C			
	65	55 sec	–	
Cycling2	95	15 sec	–	40
	60	25 sec	–	
	65	40 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	



Also **universal program** for amplification and detection "AmpliSens-1 RG" can be used (see Table 12). This program allows any combinations of tests simultaneously on one device and with unified program (for example, combined with tests for STD pathogens DNA detection).

Analytic features of the reagent set while using the universal amplification program are the same.

Table 12

Amplification program "AmpliSens-1 RG"				
Step	Temperature, °C	Time	Fluor. measurement	No of repeats
Hold	95	15 min	–	1
Cycling	95	5 sec	–	5
	60	20 sec	–	
	72	15 sec	–	
	95	5 sec	–	
Cycling2	60	20 sec	FAM/Green, JOE/Yellow, ROX/Orange, Cy5/Red	40
	72	15 sec	–	

For "iQ.iCycler" TM run one of the two following programs. For detailed programming refer to Appendix 5.

Table 13

Amplification program for HCR HPV 16, 18, 31, 33, 35; 39, 45, 51, 52, 56, 58, 59 types DNA				
Step	Temp	Time	Fluor. measurement	No of repeats
1	95 °C	15 min	–	1
2	95 °C	15 sec	–	6
	65 °C	55 sec	–	
	Temp -: 1.0 for a cycle			
3	65 °C	25 sec	–	41
	95 °C	15 sec	–	
	60 °C	55 sec	Real-time	
	65 °C	25 sec	–	



Also **universal program** for amplification and detection "AmpliSens-1 iQ" can be used (see Table 14). This program allows any combinations of tests simultaneously on one device and with unified program (for example, combined with tests for STD pathogens DNA detection).

Analytic features of the reagent set while using the universal amplification program are the same.

Table 14

Amplification program "AmpliSens-1 iQ"				
Step	Temp	Time	Fluor. measurement	No of repeats
1	95 °C	15 min	–	1
2	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
3	95 °C	5 sec	–	40
	60 °C	30 sec	FAM, HEX, ROX, Cy5	
	72 °C	15 sec	–	

For "Mx3000P" TM run the following programs. For detailed programming refer to Appendix 6.

Table 15

Amplification program for HCR HPV 16, 18, 31, 33, 35; 39, 45, 51, 52, 56, 58, 59 types DNA				
Step	Temperature, °C	Time	Fluor. measurement	No of repeats
Segment 1.	95	15 sec	–	1
Segment 2.	65	2 min	–	1
Segment 3. (Cycling)	95	20 sec	–	5
	64	25 sec	–	
	Touchdown: 1 deg. per cycle			
Segment 4. (Cycling)	65	55 sec	–	40
	95	20 sec	–	
	60	25 sec	–	
	65	55 sec	Cy5, FAM, HEX, ROX	



Also **universal program** for amplification and detection "AmpliSens-1 Mx" can be used (see Table 16). This program allows any combinations of tests simultaneously on one device and with unified program (for example, combined with tests for STD pathogens DNA detection).

Analytic features of the reagent set while using the universal amplification program are the same.

Table 16

Amplification program "AmpliSens-1 Mx"				
Step	Temp	Time	Fluor. measurement	No of repeats
Segment 1.	95 °C	15 min	–	1
Segment 2. (Cycling)	95 °C	5 sec	–	5
	60 °C	20 sec	–	
	72 °C	15 sec	–	
Segment 3. (Cycling)	95 °C	5 sec	–	40
	60 °C	30 sec	FAM, HEX, ROX, Cy5	
	72 °C	15 sec	–	

8. DATA ANALYSIS

For data processing refer to Appendix 1 (for two channel Rotor-Gene™), for Appendix 2 (for iQ.iCycler™), for Appendix 3 (for SmartCycler™), Appendix 4 (for four channel Rotor-Gene™), for Appendix 5 (for iQ5™), for Appendix 6 (for Mx3000P™).

Signal in the tube on the channel is considered to be positive, if the corresponding fluorescence accumulations 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 program of automated registration of results analyzes precisely the values of threshold cycles. According to these values the calibration curve is automatically plotted and human and HPV DNA concentrations are calculated. The final result of HPV DNA concentration normalized on number of human genomes is calculated according to the formula:

$$\lg(\text{HPV DNA copies/human DNA copies} \times 200000) = \lg(\text{HPV on } 100\,000 \text{ cells})$$

Each series of reagent set is provided with calibrators' concentrations values. These values are listed in Appendix 4, and must be noted in the corresponding cells of the program of automated analysis of results.

The reaction as a whole is **valid** if:

- Negative controls have no signal on all channels (FAM/Green, JOE/Yellow/HEX/TET);
- All calibrators have signals on all channels (FAM/Green, JOE/Yellow/HEX/TET);
- Correlation coefficient of calibration lines for all channels is no less than 0.98.

The result of HPV DNA detection of the current sample is considered to be:

Negative, if signal of internal control (IC; FAM/Green channel) is registered in both tubes for the sample, and quantity of human genomes per reaction exceeds 10^3 .

Positive, if signal on JOE/Yellow/HEX/TET channel is registered even in one of the two tubes. Result:

- One or several types from the phylogenetic group A9 (if the signal is found in tube with HPV FRT A9 mixture);
- One or several types from the phylogenetic group A7 or types 51/56 (if the signal is found in tube with HPV FRT A7+ mixture).

9. TROUBLESHOOTING.

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

- If no positive signals on JOE/Yellow/HEX/TET channel (A9, A7, A5/A6) are registered, internal control signal (IC; FAM/Green channel) is not registered and quantity of human genomes per reaction does not exceed 10^3 . In this case results of the analysis for all samples are considered invalid. It is required to repeat the analysis of all tests, and to take measures to detect and eliminate the source of contamination.
- Weak positive signal(s) is (are) registered on JOE/Yellow/HEX/TET channel, but internal control signal (IC; FAM/Green channel) is not registered and quantity of human genomes per reaction does not exceed 10^3 .

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

10. STABILITY AND STORAGE.

All components of the **AmpliSens[®] HPV HCR screen-titre-FRT** PCR kit (except for PCR-mix-1-FRT HPV A9, PCR-mix-1-FRT HPV A7+, PCR-mix-1-FRT HPV screen-titre, Polymerase (TaqF)) are to be stored at the temperature between 2 °C and 8 °C when not in use. All components of the **AmpliSens[®] HPV HCR screen-titre-FRT** PCR kit are to be stable until labeled expiration date.



PCR-mix-1-FRT HPV A9, PCR-mix-1-FRT HPV A7+, PCR-mix-1-FRT HPV screen-titre, Polymerase (TaqF) the temperature not more than minus 16 °C

11. SPECIFICATIONS.

11.1. Sensitivity.

Analytical Sensitivity of AmpliSens[®] HPV HCR screen-titre-FRT PCR kit is no less than 5×10^3 genome equivalents per 1 ml of sample for 16,18,31,35,39,45,51,52,56 and 59 types, and no less than $2,5 \times 10^4$ genome equivalents per 1 ml of sample for 33 and 58 HPV types.



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

11.2. Specificity.

Specificity of AmpliSens[®] HPV HCR screen-titre-FRT PCR kit is ensured by selection of specific primers and strict reaction conditions as well as laboratory and 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 accordance with Federal State Institution of Science "Central Research Institute of Epidemiology" ISO 13485 – certified Total Quality Management System, each lot of AmpliSens[®] HPV HCR screen-titre-FRT PCR kit is tested against predetermined specifications to ensure consistent product quality.

14. EXPLANATION OF SYMBOLS.



Manufacturer



Use by



For *in Vitro* Diagnostic Use



Catalogue number



Contains sufficient for <n> tests



Consult instructions for use



For working with Rotor-Gene™ 3000/6000



Positive control



Temperature limitation



Batch code



Version



Internal Control complex



Authorized representative in the European Community.



Caution, consult accompanying documents



For working with iQ5, iQ, iQCycler



Negative control