AmpliSens® *HPV* HCR genotype-FRT PCR kit

IVD

For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

REF	Catalogue number	⚠	Caution
LOT	Batch code	Σ	Sufficient for
IVD	*In vitro* diagnostic medical device		Use-by Date
VER	Version		Consult instructions for use
	Temperature limit		Keep away from sunlight
	Manufacturer	NCA	Negative control of amplification
	Date of manufacture	C–	Negative control of extraction
EC REP	Authorized representative in the European Community	C+	Positive control of amplification
		IC	Internal control

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 Papillomavirus* (*HPV*) types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 DNA in the clinical material (cervical and urethral swabs) using real-time hybridization-fluorescence detection of amplified products.

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

2. PRINCIPLE OF PCR DETECTION

HPV HCR detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific primers. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

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. The DNA target selected as an endogenous internal control is a human genome fragment. It must be always present in the sample (cervical swab) in sufficient quantities equivalent to the number of cells in the swab (10^3–10^5 genome equivalents). Thus, the use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and PCR amplification) but also to assess the adequacy of sampling and storage of clinical material. If epithelial swab was taken incorrectly (the number of epithelial cells is insufficient), the amplification signal of β-globin gene will be underestimated.

AmpliSens® *HPV* HCR genotype-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by the separation of nucleotides and Taq-polymerase by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The results of amplification are registered in the following fluorescence channels:

Table 1

Channel for fluorophore	FAM	JOE	ROX	Cy5
PCR-mix-1-FRT	**DNA-target**			
HPV 16/18/31	DNA of *HPV* HCR type 16	DNA of *HPV* HCR type 31	DNA of *HPV* HCR type 18	IC DNA
HPV 39/45/59	DNA of *HPV* HCR type 39	DNA of *HPV* HCR type 45	DNA of *HPV* HCR type 59	IC DNA
HPV 33/35/ 56	DNA of *HPV* HCR type 33	DNA of *HPV* HCR type 35	DNA of *HPV* HCR type 56	IC DNA
HPV 51/52/58	DNA of *HPV* HCR type 58	DNA of *HPV* HCR type 52	DNA of *HPV* HCR type 51	IC DNA
PCR-mix-1-FRT	**Target gene**			
HPV 16/18/31	gene E6	gene E6	gene E7	β–globin gene
HPV 39/45/59	gene E7	gene E6	gene E6	β–globin gene
HPV 33/35/ 56	gene E6	gene E6/E7	gene E1	β–globin gene
HPV 51/52/58	gene E6	gene E7	gene E7	β–globin gene

3. CONTENT

AmpliSens® *HPV* HCR genotype-FRT PCR kit is produced in 1 form:
variant FRT REF R-V25(RG,iQ,Mx)-CE.

Variant FRT includes:

Reagent	Description	Volume, ml	Quantity
PCR-mix-1-FRT *HPV* 16/18/31	clear liquid from colorless to light lilac colour	0.08	6 blue cap tubes
PCR-mix-1-FRT *HPV* 39/45/59	clear liquid from colorless to light lilac colour	0.08	6 pink cap tubes
PCR-mix-1-FRT *HPV* 33/35/56	clear liquid from colorless to light lilac colour	0.08	6 green cap tubes
PCR-mix-1-FRT *HPV* 51/52/58	clear liquid from colorless to light lilac colour	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 (C+$_{HPV\ 16,18,31}$)	colorless clear liquid	0.06	1 tube
Positive Control DNA *HPV* types 39, 45, 59 and human DNA (C+$_{HPV\ 39,45,59}$)	colorless clear liquid	0.06	1 tube
Positive Control DNA *HPV* types 33, 35, 56 and human DNA (C+$_{HPV\ 33,35,56}$)	colorless clear liquid	0.06	1 tube
Positive Control DNA *HPV* types 51, 52, 58 and human DNA (C+$_{HPV\ 51,52,58}$)	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 the extraction procedure as Negative Control of Extraction

AmpliSens® *HPV* HCR genotype-FRT PCR kit is intended for 108 tests (including controls)

PCR kit also includes:

Compact Disk with:
- software (Microsoft® Excel format) for data interpretation and result analysis obtaining.
- template files for fast run of experiment**.**

4. ADDITIONAL REQUIREMENTS

- DNA extraction 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 a rotor for 2 ml tubes.
- PCR box.
- Real-time instruments (for example, Rotor-Gene 3000/6000 (Corbett Research, Australia); iCycler iQ5 (Bio-Rad, USA); Mx3000P (Stratagene, USA); CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene PCR tubes (0.1- or 0.2-ml) or strips of eight 0.2-ml tubes with optical transparent caps:
  a) 0.1-ml PCR tubes if Rotor-Gene instrument is used;
  b) 0.2-ml PCR tubes with domed caps or strip tubes or plates for PCR with optical transparent thermostable adhesive films if Mx3000P (Stratagene, USA) instrument is used.
  c) 0.2-ml PCR tubes with optical transparent domed caps or strip tubes with domed cap or 96-well plate for PCR with optical transparent adhesive films if iCycler iQ5 instrument is used.
- Refrigerator at the temperature from 2 to 8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. 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.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- Safety Data Sheets (SDS) are available on request.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.

NOTE: Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

NOTE: 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.

AmpliSens® *HPV* HCR genotype-FRT PCR kit is intended for analysis of the DNA extracted with DNA extraction kits from the biological material (cervical and urethral swabs).

Female: samples of epithelial cells should be obtained as for cytological examination:

Method 1 The sampling kit with one/two cervical cytobrushes and 2.0-ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** REF 953-CE are used.

Place the cervical epithelial scrape (endocervix) taken with the first cervical cytobrush and/or the superficial cervical scrape (ectocervix) taken with the second cervical cytobrush to the tube with transport medium. Snap off the lower part of the cytobrush and leave it in the tube with transport media.

Method 2 The Digene Cervical Sampler (USA), which contains cervical cytobrush and a tube with 1.0-ml of Digene transport medium.
Place the cervical epithelial scrape (endocervix) obtained with cytobrush into the tube with Digene transport medium.

Method 3 The sampling kit, which contains the combined gynecological probe for simultaneous taking of epithelium from endo-/exocervix and 5.0-ml tube with 2.0 ml of **Transport Medium with Mucolytic Agent** REF 953-CE is used.

Place the cervical epithelial scrape (endocervix) and superficial cervical scrape (ectocervix) into the tube with the transport medium. Snap off the lower part of the probe and leave it in the tube with transport medium.

Method 4 The sampling kit, which contains a combined gynecological probe for simultaneous taking of epithelium from endo-/exocervix and a vial with CytoScreen (Italy) or PreservCyt (USA) preservation/transportation medium for fluid cytology is used.
Place the cervical epithelial scrape (endocervix) and superficial cervical scrape (ectocervix) into the tube with the transport-fixation medium. Snap off the lower part of the probe and leave it in the vial with transport medium.

Male: Obtain urethral epithelial scrape by universal probe and place it into the 2.0 ml tube with 0.5 ml of **Transport Medium with Mucolytic Agent** REF 953-CE.

The biological samples can be stored:

- at the temperature from 18 to 25 °C – no more than 5 days;
- at the temperature from 2 to 8 °C – no more than 20 days;
- at the temperature below minus 16 °C – for 1 year. Only one freeze-thawing cycle is allowed;
- in the transport medium for liquid-based cytology at room temperature– for 1 year.

7. WORKING CONDITIONS

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

8. PROTOCOL

8.1. DNA Extraction

It is recommended to use the following nucleic acid extraction kits:

- **DNA-sorb-AM**, REF K1-12-100-CE (for clinical material obtained by the 1st, 2nd and 3rd methods);
- **DNA-sorb-B**, REF K1-2-100-CE (for clinical material obtained by the 1st, 2nd and 3rd methods);
- **DNA-sorb-C**, REF K1-6-50-CE (for biopsy of mucous).
- **AmpliSens® DNA-sorb-D**, REF K8-2331-100-CE (for liquid-based cytology samples)

In the extraction procedure it is necessary to carry out the control reactions as follows:

C– – Add **100 µl of Negative Control (C–)** to the tube labelled C– (Negative Control of Extraction).

NOTE: Extract DNA according to the manufacturer's instructions.

NOTE: In case of extracting with the DNA-sorb-AM reagent kit, don't add **Internal Control complex (ICc)** or **Internal Control-FL (IC)**.

8.2. Preparing PCR

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

8.2.1 Preparing tubes for PCR

1 Prepare the mixture of **PCR-buffer-FRT** and **polymerase (TaqF).** To do this, transfer the whole content of the tube with **polymerase (TaqF) (60 µl)** into the tube with **PCR-buffer-FRT (1100 µl)**. Carefully vortex the tube avoiding foaming. Indicate the date of mixture preparation on the tube.

NOTE: This mix is intended for analysis of 54 samples, including controls (3 times with 18 samples or three full load of Rotor-Gene rotor).
Store the mixture at 2-8 ºC for 3 months and use as required.

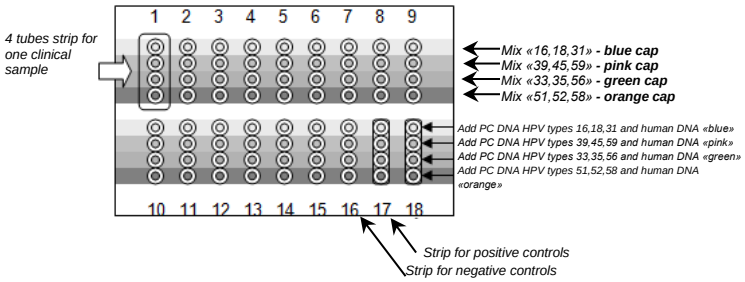
2 **For genotyping of 16 clinical samples**:
- take the required number of tubes/strips for amplification of the DNA obtained from clinical and control samples (strips of four tubes: 16 strips for samples + 2 control strips; strips of eight tubes: 8 strips for samples + 1 control strip),
- prepare one tube of each of the four PCR-mix-1-FRT HPV,
- add 90 µl of prepared mixture of polymerase (TaqF) and PCR-buffer-FRT into each of the four PCR-mix-1-FRT HPV tubes (with blue, pink, green, orange caps) and carefully vortex the tubes avoiding foaming.

If it is necessary to test **less than 16 samples** for N reactions, add to a new tube:
3.5*(N+2) µl of each **PCR-mix-1**,
4.5*(N+2) µl of the mixture of polymerase (TaqF) and **PCR-buffer-FRT**.
For example, for 8 samples and 2 controls (Positive and Negative) prepare 35 µl of each PCR-mix-1 (3.5[8+2]) and add 45 µl of mixture of polymerase (TaqF) and PCR-buffer-FRT (4,5*[8+2]).*

NOTE: *The reaction mixture (containing PCR-mixes-1, polymerase (TaqF) and PCR-buffer-FRT) should be used within 2 hours.*

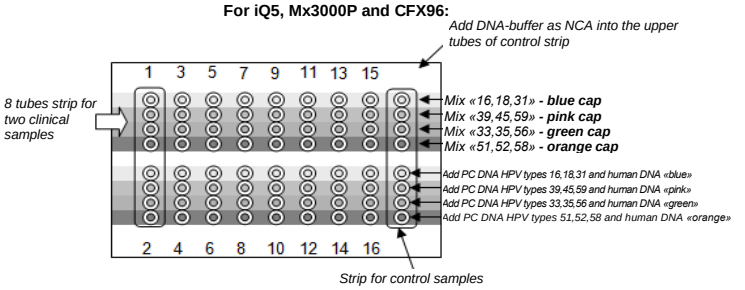
3 Transfer **8.0 µl** of reaction mixture into each tube. Dispense reagents and samples as shown below (every sample has to be tested in 4 tubes): Add to the first strip tube **8.0 µl** of mix "16,18,31" (blue cap), to the second strip tube **8.0 µl** of mix "39,45,59" (pink cap), to the third strip 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.

For Rotor-Gene 3000/6000:



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, Mx3000P and CFX96:



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

NOTE: Do not to change the reaction mixes order in strips for adequate results processing.

Add **5 µl** of **extracted DNA samples** into 4 tubes with different reaction mixtures using tips with aerosol filter.

NOTE: Avoid transferring the sorbent together with the DNA samples.

4 Carry out the control amplification reactions:

NCA or C– – Add **5 µl** of **DNA-buffer** into 4 tubes with different reaction mixtures (Negative Control of Amplification)
or
– Add **5 µl** of **the sample extracted from the Negative Control (C–) reagent** into 4 tubes with different reaction mixtures (Negative Control of Extraction).

C+*HPV* 16,18,31 – Add **5 µl** of **Positive Control DNA *HPV* types 16, 18, 31 and human DNA** into the tube with "16,18,31" reaction mixture;

C+*HPV* 39,45,59 – Add **5 µl** of **Positive Control DNA *HPV* types 39, 45, 59 and human DNA** into the tube with "39,45,59" reaction mixture;

C+*HPV* 33,35,56 – Add **5 µl** of **Positive Control DNA *HPV* types 33, 35, 56 and human DNA** into the tube with "33,35,56" reaction mixture;

C+*HPV* 51,52,58 – Add **5 µl** of **Positive Control DNA *HPV* types 51, 52, 58 and human DNA** into the tube with "51,52,58" reaction mixture;

8.2.2. Amplification

1. Create a temperature profile on your instrument as follows:

Table 2

For Rotor-Gene: RG amplification program

Step	Temperature, °C	Time	Cycles
1	95	15 min	1
2	95	15 s	45
	60	30 s Fluorescence detection	

Table 3

For iCycler iQ5: IQ amplification program
Amplification program for CFX96

Step	Temperature, °C	Time	Cycles
1	95	15 min	1
2	95	15 s	45
	60	50 s Fluorescence detection	

Table 4

For Mx3000P: Mx amplification program

Step	Temperature, °C	Time	Cycles
1	95	15 min	1
2	95	20 s	45
	60	60 s Fluorescence detection	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX and Cy5 fluorophores

NOTE: AmpliSens-1 universal amplification program also can be used. Any combination of the tests (for example, with the tests for detection of STI pathogens DNA) can be performed in one instrument simultaneously with the use of the universal amplification program. The analytical features of the PCR kit do not change with the use of AmpliSens-1 universal program.

Table 5

AmpliSens-1 amplification program

	Rotor-type Instruments[1]			Plate-type Instruments[2]		
Step	Temperature, °C	Time	Cycles	Temperature, °C	Time	Cycles
1	95	15 min	1	95	15 min	1
2	95	5 s	5	95	5 s	5
	60	20 s		60	20 s	
	72	15 s		72	15 s	
3	95	5 s	40	95	5 s	40
	60	20 s *Fluorescence acquiring*		60	30 s *Fluorescence acquiring*	
	72	15 s		72	15 s	

Fluorescent signal is detected in the channels for the FAM, JOE, ROX and Cy5 fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run)

2. Adjust the fluorescence channel sensitivity according to the Guidelines [2]..
3. Insert tubes into the reaction module of the device.
4. Run the amplification program with fluorescence detection.
5. Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Refer to the Guidelines [2] for data analysis.

Signal in the tube in a given channel is considered positive if the corresponding fluorescence accumulation curve crosses the threshold line. The characteristic of a given signal is the threshold cycle – the cycle that correspond to the crossing point of fluorescence curve and threshold line. The threshold cycles values (and also its presence or absence) are analyzed by the software of automatic result interpretation.

The experiment is ***valid*** if:

- for the negative controls the positive signal is absent in all channels all the channels for the fluorophores (FAM, JOE, ROX, Cy5);
- for the positive control all 12 genotypes of *HPV* are detected.

NOTE: In case of invalid experiment all obtained data are considered unreliable. The experiment is to be repeated.

The result of qualitative detection and genotyping *HPV* DNA for the sample is considered**.**

- *invalid,* if no positive signal is detected on any channel in any strip tube, including IC channel (Cy5). (For Rotor-Gene only: a sample is also considered to be invalid if only the IC signal is detected and the *Ct* value for this channel exceeds 35 if running the RG amplification program or 30 if running the Amplisens-1 program).
- *negative* if in all four tubes of strip the signal of internal control is present (Cy5 channel) and the signals are absent in other channels for the fluorophores (FAM, JOE, ROX).
- *positive,* in all other cases.

NOTE: The absence of internal control signal (Cy5 channel) in the tube of strip is acceptable if the signal/signals is/are detected in this tube in the channels for the FAM, JOE, ROX fluorophores.

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

NOTE: 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.*

The absence of the IC signal (channel for the Cy5 fluorophore) in a strip tube is acceptable if signal/signals in the channels for the FAM, JOE and ROX fluorophores is/are detected and *Ct* values do not exceed 35 if running the RG amplification program or 30 if running the AmpliSens-1 program..

10. TROUBLESHOOTING

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

1. If any *Ct* value appears for Negative Controls (C–, NCA) on any channel, it indicates reagent or sample contamination. In this case results of the analysis for all samples are considered invalid. It is necessary to repeat the analysis of all tests, and measures for detecting and eliminating the contamination source must be taken.
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 8.3), 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.

11. TRANSPORTATION

AmpliSens® *HPV* HCR genotype-FRT PCR kit should be transported at 2–8 °C for no longer than 5 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® *HPV* HCR genotype-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for PCR-mix-1-FRT *HPV* 16/18/31, 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)). All components of the **AmpliSens® *HPV* HCR genotype-FRT** PCR kit are to 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.

NOTE: PCR-mix-1-FRT *HPV* 16/18/31, 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 the temperature from minus 24 to minus 16 ºC.

NOTE: PCR-mix-1-FRT *HPV* 16/18/31, PCR-mix-1-FRT *HPV* 39/45/59, PCR-mix-1-FRT *HPV* 33/35/56, and PCR-mix-1-FRT *HPV* 51/52/58 are to be kept away from light.

13. SPECIFICATIONS

13.1. Sensitivity

Analytical sensitivity of **AmpliSens® *HPV* HCR genotype-FRT PCR kit** is no less than $1x10^3$ genome equivalents per 1 ml of sample for 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 types.

NOTE: The claimed analytical performance characteristics of **AmpliSens® *HPV* HCR genotype-FRT** PCR kit are guaranteed only when additional reagent kits **DNA-sorb-B**, **DNA-sorb-AM**, **DNA-sorb-C**, **AmpliSens® DNA-sorb-D** (manufactured by Federal Budget Institute of Science "Central Research Institute for Epidemiology") are used.

13.2. Specificity

The analytical specificity of **AmpliSens® *HPV* HCR genotype-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The clinical specificity of **AmpliSens® *HPV* HCR genotype-FRT** PCR kit was confirmed in laboratory clinical trials.

14. REFERENCES

1. Handbook "Sampling, Transportation, and Storage of Clinical Material for PCR Diagnostics" developed by Federal State Institute of Science "Central Research Institute for Epidemiology" of Federal Service for Surveillance on Consumers' Rights Protection and Human Well-Being.
2. Guidelines to the **AmpliSens® *HPV* HCR genotype-FRT** PCR kit 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 by polymerase chain reaction (PCR) with 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* HCR genotype-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
20.01.11	Cover page	The phrase “For Professional Use Only” was added
20.01.11	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”
	Key to Symbols Used	The explanation of symbols was corrected
	Through the text	For Positive Controls Positive Control DNA *HPV* types 16, 18, 31 and human DNA, Positive Control DNA *HPV* types 39, 45, 59 and human DNA, Positive Control DNA *HPV* types 33, 35, 56 and human DNA, Positive Control DNA *HPV* types 51, 52, 58 and human DNA abbreviations $C+_{16,18,31}$, $C+_{39,45,59}$, $C+_{33,35,56}$, $C+_{51,52,58}$ were added
		Writing of causative agent *HPV* is changed to italic
22.06.11 RT	Cover page, text	The name of Institute was changed to Federal Budget Institute of Science “Central Research Institute for Epidemiology”
12.07.12 LA	9. Data analysis	Ct values were changed throughout the text
21.12.12 LA	3. Content 8. Protocol	Short designations of the positive controls of amplification were corrected: $C+_{HPV\ 16,18,31}$, $C+_{HPV\ 39,45,59}$, $C+_{HPV\ 33,35,56}$, $C+_{HPV\ 51,52,58}$ instead of $C+_{16,18,31}$, $C+_{39,45,59}$, $C+_{33,35,56}$, $C+_{51,52,58}$, respectively
16.04.20 KK	Through the text	The text formatting was changed
	Footer	The phrase “Not for use in the Russian Federation” was added
14.04.14 ME	Text	Changes in accordance with the template and Russian instruction manual
	8.3. Amplification	The amplification programs for CFX96 were added
01.02.16 PM	Through the text	Corrections according to the template
	8.1. DNA extraction	AmpliSens® DNA-sorb-D nucleic acid extraction kit was added
	8.2.2 Amplification	The section was rewritten
13.02.18 ME	Text	The reference number of AmpliSens® DNA-sorb-D nucleic acid extraction kit was changed
05.09.18 EM	3. Content	The colours of the reagents were specified
16.11.18 EM	5. General precautions	The section was corrected according to the template
26.11.18 EM	3. Content	The information about the content of Compact Disk was added
15.04.20 KK	Through the text	The text formatting was changed
	2. Principle of PCR detection	The table with targets was added
	Footer	The phrase “Not for use in the Russian Federation” was added
24.03.21 EM	—	The name, address and contact information for Authorized representative in the European Community was changed

AmpliSens®

EC REP

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