ECOLI

eSens

CE

IVD

For Professional Use Only

eSens HPV LR 6/11 genotype QL PCR kit

REF ES3090A

Instructions for Use

1 INTENDED USE

eSens HPV LR 6/11 genotype QL PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection and differentiation of genotypes 6 and 11 of *human papillomavirus* (*HPV*) DNA in the clinical material (urogenital 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 genotypes 6 and 11 detection by the polymerase chain reaction (PCR) is based on the amplification of the pathogen genome specific region using specific *HPV* 6/11 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.

Principle of testing is based on simultaneous amplification (multiplex PCR) of DNA fragments of *HPV* and a fragment of β-globin gene which is used as an endogenous internal control. PCR analysis for *HPV* types 6 and 11 DNA detection is carried out in one tube. The DNA-target selected as an endogenous internal control is a human genome fragment. It must be present in a sample (cervical scrape) in sufficient amount equivalent to the number of cells in the sample (10^5-10^7 cells/ml). Thus, the use of an endogenous internal control makes it possible not only to monitor test stages (DNA extraction and 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 weak.

eSens HPV LR 6/11 genotype QL PCR kit uses “hot-start”, which greatly reduces the frequency of nonspecifically primed reactions. “Hot-start” is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

HPV genotypes 6 and 11 are low carcinogenic risk viruses that are not associated with cervical carcinoma. Low–risk *HPV* types have a productive effect on cells. These viruses are responsible for onset of genital warts (genital pointed condyloma), recurrent respiratory throat papillomatosis; and more than 95% of these pathologies are associated with genotypes 6 and 11.

Necessary to mention, that genotypes 6 and 11 are the part of quadrivalent vaccine which protects from *HPV* 6/11/16/18 infections. Therefore, detection of *HPV* types 6 and 11 can help in evaluating of the efficacy of vaccinal prevention from cervical cancer and benign genital tumors.

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

Table 1

Channel for fluorophore	**FAM**	**JOE**	**ROX**
DNA-target	*HPV* genotype 6 DNA	*HPV* genotype 11 DNA	IC DNA
Target gene	*gene* E6	*gene* E7	DNA fragment of β-globin gene

3 CONTENT

eSens HPV LR 6/11 genotype QL PCR kit (ES3090A) includes:

Reagent	**Description**	**Volume, ml**	**Quantity**
PCR-mix-1-FL *HPV* 6/11	colorless clear liquid	0.3	4 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 extraction procedure as Negative Control of Extraction

eSens HPV LR 6/11 genotype QL PCR kit is intended for 110 reactions, including controls.

4 ADDITIONAL REQUIREMENTS

- Transport medium.
- DNA extraction kit.
- Sterile pipette tips with aerosol filters (up to 200 µl).
- Tube racks.
- Vortex mixer.
- Desktop centrifuge with rotor for 2-ml reaction tubes.
- PCR box.

- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), CFX 96 Touch, CFX 96 Opus (Bio-Rad, USA), QuantStudio 5 (Thermo Fisher Scientific), or equivalent).
- Disposable polypropylene tubes
    - 0.2-ml PCR tubes with domed caps if a plate-type instrument is used;
    - 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Reservoir for used tips.
- Disposable powder-free gloves and a laboratory coat.

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.

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

6 SAMPLING AND HANDLING

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

Method 1. The sampling kit, which contains the combined gynecological probe for simultaneously taking epithelium from endocervix and ectocervix and 5-ml tube with 2.0 ml of **Transport Medium with Mucolytic Agent** (952-CE), is used.

Place the endocervix and ectocervix into the tube with transport medium. Break the lower part of the probe and leave it in the tube with transport medium.

Method 2. The Digene cervical sampler (USA), which contains cervical cytobrush and a tube with 1.0 ml of Digene transport medium, is used.

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 simultaneously taking epithelial samples from endocervix and ectocervix and a liquid-based cytology vial with CytoScreen (Italy) or PreservCyt (USA) transport medium, is used.

Place the endocervical and exocervical into the tube with transport medium. Break the lower part of the probe and leave it in the vial with transport medium.

Male: Obtain urethral epithelial scrape by universal probe, place it into the 2-ml tube with 0.5 ml of Transport Medium with Mucolytic Agent

Storage conditions:

- 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 from - 24 to - 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

eSens HPV LR 6/11 genotype QL PCR kit should be used at 18–25 °C.

8 PROTOCOL

8.1 DNA extraction

Ecoli Dx, s.r.o. recommends:

- For the manual extraction

- **DNA-sorb-AM** (K1-12-100-CE)

- For the automatic extraction

- **ePure STD DNA Extraction Kit (E2007)**

Please carry out nucleic acid extraction according to the manufacture´s instruction.

8.2 Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis. Use disposable filter tips for adding reagents, DNA and control samples into tubes.

The total reaction volume is 25 µl, the volume of DNA sample is 10 µl.

1. Prepare the mixture of PCR-mix-2-FRT and polymerase (TaqF). To do this, transfer the content of one tube with polymerase (TaqF) (30 µl) to the tube with PCR-mix-2-FRT (300 µl). Vortex carefully to avoid foaming. Indicate the date of mixture preparation on the tube.

NOTE: The prepared mixture is intended for analysis of 60 samples. The mixture can be stored at 2–8 °C for up to 3 month and used as required.

2. Prepare the reaction mixture (see Table 2). When calculating the volume of the mixture, take into account the necessity to run three control reactions. Do not forget to add extra volumes for one more reaction.

   Each PCR reaction requires:

   - 10 µl of PCR-mix-1-FL *HPV* 6/11
   - 5 µl of the mixture of PCR-mix-2-FRT and polymerase (TaqF).

Table 2

Scheme of reaction mixture preparation

Number of test samples	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
PCR-mix-1-FL *HPV* 6/11, µl	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220
Mixture of PCR-mix-2-FRT and polymerase (TaqF), µl	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110
Number of test samples	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**
PCR-mix-1-FL *HPV* 6/11, µl	230	240	250	260	270	280	290	300	310	320	330	340	350	360	370
Mixture of PCR-mix-2-FRT and polymerase (TaqF), µl	115	120	125	130	135	140	145	150	155	160	165	170	175	180	185

Note – The calculation scheme for samples is given according to formula n + 4, where n is the number of clinical samples for analysis; 4 is the controls of PCR analysis (1 Control of Extraction, 2 Controls of amplification, and 1 extra tube).

3. Prepare the required number of tubes for amplification of DNA from clinical and control samples. Transfer 15 µl of the prepared mixture into each tube.
4. Add 10 µl of the DNA samples obtained at the DNA extraction stage.
5. Carry out 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 (C+$_{HPV\ 6,11}$)** to the tube labeled C+ (Positive Control of Amplification).
C–	Add **10 µl** of the sample extracted from the **Negative Control (C–)** reagent to the tube labeled C– (Negative Control of Extraction).

8.2.2 Amplification

1. Create a temperature profile on your instrument as follows (see Table 3):

Table 3

eSens-1 amplification program

	Rotor-type Instruments (e.g Rotor-Gene Q or equivalent)			**Plate-type Instruments** (e.g CFX 96 Touch, CFX 96 Opus, QuantStudio 5 or equivalent.)		
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 *fluorescent signal detection*		60	30 s *fluorescent signal detection*	
	72	15 s		72	15 s	

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

2. Adjust the fluorescence channel sensitivity.
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.

8.3 Instrument Settings

Test settings for rotor-type instruments

Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q

Channel	Calibrate/Gain Optimisation	Threshold	Dynamic tube	Slope Correct	More Settings/ Outlier Removal
FAM/Green	from 4 Fl to 8 Fl	0.03	On	On	10%
JOE/Yellow	from 4 Fl to 8 Fl	0.03	On	Off	10%
ROX/Orange	from 4 Fl to 8 Fl	0.03	On	On	10%

Test settings for plate-type instruments

iCycler iQ, iQ5, Mx3000P, Mx3005P, CFX96

iCycler iQ5

Channel	Threshold
FAM JOE/HEX ROX	In the *Base Line Threshold* window set the *Base Line Cycles - Auto Calculated* parameter (if cut, set this parameter in the *User Defíned,* 2 *through 10 cycles* mode) and set the *Crossing Threshold-Auto Calculated* parameter. Normally, a threshold line should cross only S-shaped curves of positive samples and controls and should not cross a base line.

iCycler iQ

Channel	Threshold
FAM JOE/HEX ROX	In the *Threshold Cycle Calculation* menu indicate that threshold line adjusting and base line calculation are carried aut in automatic mode. To do this, select *Auto Calculated in* the *Baseline Cycles* submenu and select *Auto Calculated in* the *Threshold Position* submenu. Normally, a threshold line should cross only S-shaped curves of signal accumulation of positive samples and controls and should not cross the base line.

Mx3000P

Channel	Threshold
FAM JOE/HEX ROX	Select the *Threshold fluorescense* field and make sure that tick marks are put against three fluorescence channels: JOE/HEX, FAM and ROX. Ensure that the threshold line is set correctly. Normally, a threshold line should cross S-shaped curves of signal accumulation of positive samples and controls and should not cross the base line.

CFX96

Note - Set ***Ramp Rate* 2,5 °C/s** by clicking the *Step Options* button for each step of cycling.

Channel	**Threshold**
FAM JOE/HEX ROX	**Variant 1:** For each channel at a time set the threshold line at the level of 10-20 % of maximum fluorescence obtained for the Positive Controls in the last amplification cycle. Make sure that fluorescence curve of the Positive Control crosses the threshold line at the zone of exponential growth of fluorescence passing onto linear growth. **Variant 2:** For each channel at a time tick off log Scale. Set the threshold line at the level, where the fluorescence curves are linear f and above the noise level.

9 DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in three channels:

- The signal of the *HPV* type 6 DNA amplification product is detected in the channel for FAM fluorophore.
- The signal of the *HPV* type 11 DNA amplification product is detected in the channel for JOE fluorophore.
- The signal of the internal endogenous control (IC) β-globin DNA amplification product is detected in the channel for ROX fluorophore.

Channel	**FAM**	**JOE**	**ROX**
Result	*HPV* 6	*HPV* 11	IC

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a *Ct* value of the DNA sample in the corresponding column of the results grid.

Principle of interpretation is the following (see Table 4):

- ***HPV* genotype 6 DNA is detected** if the *Ct* value determined in the channel for the FAM fluorophore is less than the boundary *Ct* value
- ***HPV* genotype 11 DNA is detected** if the *Ct* value determined in the channel for the JOE fluorophore is less than the boundary *Ct* value
- ***HPV* genotypes 6 and 11 DNA are not detected** if the *Ct* value of a sample is not determined (absent) in the result grid in the channel for the FAM and JOE fluorophore, or greater than the boundary *Ct* value; whereas the *Ct* value determined in the result grid in the channel for the ROX fluorophore is less than the *Ct* value.

- The result is **invalid** if the *Ct* value of a sample in the channel for the ROX fluorophore is absent or greater than the boundary *Ct* value.

Table 4

Interpretation of results

FAM (*HPV* 6)	JOE (*HPV* 11)	ROX (IC)	Result
–	–	+	***HPV* genotypes 6 and 11 are not detected**
+	–	+	***HPV* genotype 6 is detected**
+	–	–	
–	+	+	***HPV* genotype 11 is detected**
–	+	–	
+	+	+	***HPV* genotypes 6 and 11 are detected**
+	+	–	
–	–	–	**Invalid result**

The result of the analysis is considered reliable only if the results for the Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 5 an 6).

Table 5

Results for controls

Control	**Stage for control**	***Ct* value in the channel for fluorophore**		
		FAM	**JOE**	**ROX**
C–	DNA extraction	Absent	Absent	Absent
NCA	PCR	Absent	Absent	Absent
C+	PCR	< boundary value	< boundary value	< boundary value

Table 6

Boundary *Ct* values

FAM / Green	JOE / HEX / Yellow	ROX / Orange
HPV6	*HPV 11*	*IC*
Rotor-Gene 3000, Rotor-Gene 6000, Rotor-Gene Q		
32	**32**	**32**
iCycler iQ, iCycler iQ5, CFX96		
32	**34**	**32**
Mx3000P, Mx3005P		
34	**32**	**32**

10 TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

1. If any *Ct* value is present for the Negative Control of Amplification (NCA) and/or for Negative Control of Extraction (C–) in the channels for the FAM, JOE and/or ROX fluorophores in the results grid, it indicates reagent or sample contamination. In this case, the results of analysis must be considered invalid. The analysis should be repeated starting from DNA extraction stage for all samples in which *HPV* DNA was detected and measures for detecting and eliminating the contamination source must be taken.
2. If the *Ct* value determined for the Positive Control of Amplification (C+) in the channels for the FAM, JOE and/or ROX fluorophores is greater than the boundary *Ct* value or absent, the amplification should be repeated for all samples in which *HPV* DNA was not detected.
3. If the *Ct* value of a sample is not determined or exceeds the boundary value in the channels for the FAM and/or JOE fluorophores, while *Ct* value determined in the channel for the ROX fluorophore is greater than the boundary value, PCR should be repeated starting from DNA extraction stage. It can be caused by a failure in clinical material preparation that led to DNA loss or presence of inhibitors.

11 TRANSPORTATION

eSens HPV LR 6/11 genotype QL PCR kit should be transported at 2-8 °C for no longer than 5 days.

12 STABILITY AND STORAGE

All components of the **eSens HPV LR 6/11 genotype QL PCR kit** are to be stored at 2-8 °C when not in use (except for PCR-mix-1-FL *HPV* 6/11, PCR-mix-2-FRT, and polymerase (TaqF). All components of the **eSens HPV LR 6/11 genotype QL PCR kit** are to be stable until the expiration date on the label. The shelf life of reagents before and after the first use is the same, unless otherwise stated.

NOTE: PCR-mix-1-FL HPV 6/11, PCR-mix-2-FRT, and polymerase (TaqF) are to be stored at the temperature from minus 24 to minus 16 °C.

NOTE: PCR-mix-1-FL HPV 6/11 is to be kept away from light.

13 SPECIFICATIONS

13.1 Sensitivity

Clinical material	Transport medium	DNA extraction kit	PCR kit	Analytical sensitivity, GE/ml*
Cervical epithelial swab	Transport Medium with Mucolytic Agent	**DNA-sorb-AM ePure STD DNA Extraction Kit**	variant FRT	$1x10^3$

* Number of genome equivalents of microorganism (GE) per 1 ml of clinical sample placed in the specified transport medium

13.2 Specificity

The analytical specificity of **eSens HPV LR 6/11 genotype QL 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.

eSens HPV LR 6/11 genotype QL PCR kit detects a fragment of DNA of *HPV* genotypes 6 and 11. The analytical specificity of the PCR kit was investigated by adding to the reaction DNA/RNA of different microorganisms (*adenovirus* types 2, 3 and 7; *cytomegalovirus*; *Epstein-Barr virus*; *Varicella-Zoster virus*; *hepatitis* B and C; *human immunodeficiency virus* type 1; *human herpes virus* type 6 and 8; *herpes simplex virus*; *Chlamydia trachomatis*; *Mycoplasma hominis, M.genitalium*; *Ureaplasma urealyticum*; *Gardnerella vaginalis*; *Neisseria gonorrhoeae*; *Trichomonas vaginalis*; *Candida albicans*; *Streptococcus pyogenes*; *Staphylococcus aureus*; the DNA of human papillomavirus genus β, γ, μ (1, 3, 4, 5, 8, 37, 38, 65, 20, 24, 49, 50, 15), genus α of low and unknown carcinogenicity risk (26, 53, 7, 27, 10), and genus α of high carcinogenicity risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) at a concentration of 10^8 copies of *HPV* DNA per ml). Cross-reactivity was not observed.

The clinical specificity of **eSens HPV LR 6/11 genotype QL PCR kit** was confirmed in laboratory clinical trials.

14 QUALITY CONTROL

The production process, including batch release, is carried out in accordance with an established quality management system certified according to ISO 13485.

15 KEY TO SYMBOLS USED

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

List of Changes Made in the Instruction Manual

VER	Location of changes	Essence of changes
01_04/2022		

Ecoli Dx, s.r.o. , Purkyňova 74/2

110 00 Praha 1, Česká republika
Tel: +420 325 209 912

Mobil: +420 739 802 523