











# HPV 16/18

## Key to symbols used

	List Number		Store at 2-8°C
	For Research Use Only		Caution!
	Lot Number		Version
	Expiration Date		Consult instructions for use
	Contains reagents		Manufacturer

### NAME

HPV 16/18

### INTENDED USE

HPV 16/18 is an *in vitro* nucleic acid amplification test for qualitative detection of *Human Papillomavirus genotypes 16 and 18* in the urogenital swabs.

### PRINCIPLE OF ASSAY

HPV 16/18 Test is based on three major processes: sample preparation, multiplex amplification of DNA using specific *HPV 16 and 18* primers and detection of the amplified products on agarose gel.

### MATERIALS PROVIDED

- **PCR-mix-1 "16/18"**, 55 tubes
- **PCR-mix-2**, 0,6 mL
- **Mineral Oil**, 1,0 mL
- **Controls:**
  - **HPV 16 18 DNA (C+)**, 0,2 mL
  - **Negative Control C-\***, 1,2 ml;
  - **DNA-buffer (C-)**, 0,5 mL


Contains reagents for 55 samples.

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


### MATERIALS REQUIRED BUT NOT PROVIDED

- Thermalcycler
- Workstation
- Pipettors (capacity 0,5-10 µl; 5-40 µl) with aerosol barrier
- Tube racks

### Reagents non provided

- DNA extraction kit (recommended nucleic acid extraction kit: DNA-Sorb-A (Sacace,  K-1-1/A))
- Detection agarose kit

### WARNINGS AND PRECAUTIONS

1.  Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.
2. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
3. Do not pipette by mouth.
4. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
5. Do not use a kit after its expiration date.
6. Dispose of all specimens and unused reagents in accordance with local regulations.
7. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
8. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
9. Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
10. Material Safety Data Sheets (MSDS) are available on request.
11. This kit is designed for use with "DNA-Sorb" extraction kit. It is the user's responsibility if kits other than "DNA-Sorb" are used to perform this DNA extraction.
12. Use of this product should be limited to personnel trained in the techniques of amplification.
13. Workflow in the laboratory must proceed in a uni-directional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where you performed previous step.

## STORAGE INSTRUCTIONS

HPV 16/18 must be stored at 2-8°C.

## STABILITY

HPV 16/18 is stable up to the expiration date indicated on the kit label.

## SAMPLE COLLECTION, STORAGE AND TRANSPORT

HPV 16/18 can analyze DNA extracted with **DNA-Sorb-A** (REF K-1-1/A) from:

- *Cervical swabs*: insert the swab into the nuclease-free 1,5 ml tube and add 0,2 mL of Transport medium. Vigorously agitate swabs in medium for 15-20 sec.

It is recommended to process samples immediately after collection. Store samples at 2–8 °C for no longer than 24 hours, or frozen at –20/80°C. Transportation of clinical specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

## AMPLIFICATION

1. Prepare required quantity of tubes **PCR-mix-1 “16/18”**.
2. Pipette **10 µL** of **PCR-mix-2** into each PCR-mix-1 tube.
3. Add to appropriate tube **10 µL** of **DNA** sample obtained after sample preparation.
4. Add **10 µL** of **DNA-buffer** to the tube for Negative Control of Amplification.
5. Add **10 µL** of **HPV 16 DNA (C+)** to the tube labeled C<sub>16+</sub>, **10 µL** of **HPV 18 DNA (C+)** to the tube labeled C<sub>18+</sub> for Positive Controls of Amplification.
6. Close PCR-mix-1 tubes and transfer them into the thermalcycler only when temperature reaches 95°C and start the following program:

Step	Thermocyclers with active temperature adjustment “GeneAmp PCR System 2700” (Applied Biosystems), “Gradient Palm Cycler” (Corbett Research)			Thermocyclers with block temperature adjustment: <b>Biometra</b> ”, “MiniCycler”, “PTC-100”(MJ Research)		
	t°C	Time	Cycles	t°C	Time	Cycles
0	95°C	Pause		95°C	Pause	
1	95°C	5 min	1	95°C	5 min	1
2	95°C	15 sec	42	95°C	1 min	42
	65°C	25 sec		65°C	1 min	
	72°C	25 sec		72°C	1 min	
3	72°C	1 min	1	72°C	1 min	1
4	4°C	Storage		10°C	Storage	

## RESULTS ANALYSIS

Analysis of PCR results is based on the presence or absence of specific bands of amplified DNA in Agarose gel (2%). The length of specific amplified DNA fragments is:

- **HPV 16 – 325 bp**
- **HPV 18 – 425 bp**

## RESULTS INTERPRETATION

**Table 2. Results for controls**

Control	Which step of test is controlled	Specific bands in the gel <b>325 bp</b>	Specific bands in the gel <b>425 bp</b>	Interpretation
NCS	DNA isolation	No	No	Valid result
DNA-buffer	Amplification	No	No	Valid result
HPV 16 C+	Amplification	<b>Yes</b>	No	Valid result
HPV 18 C+	Amplification	No	<b>Yes</b>	Valid result

- Ensure that the Negative Controls (NEG CONTROL and DNA-buffer) values for the run are valid. If the run is invalid (contamination – detection of the specific band, the entire test protocol (sample preparation, amplification and detection) should be repeated. Discard any reagents that may be suspect.

The sample is considered to be positive for *HPV 16 DNA* if the band of 325 bp is observed on agarose gel and positive for *HPV 18 DNA* if the band of 425 bp is.

## PERFORMANCE CHARACTERISTICS

### Analytical specificity

The analytical specificity of the primers and probes was validated with negative samples. They did not generate any signal with the specific HPV primers and probes. The specificity of the kit HPV 16/18 was 100%. The potential cross-reactivity of the kit HPV 16/18 was tested against the group control. It was not observed any cross-reactivity with other pathogens.

### Analytical sensitivity

The kit HPV 16/18 allows to detect HPV DNA in 100% of the tests with a sensitivity of not less than 500 copies/ml. The detection was carried out on the control standard and its dilutions by negative sample.

**Target region:** E6 genes



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