















For *in Vitro* Diagnostic Use



HPV High Risk Typing

Key to symbols used

	List Number		Store at 2-8°C
	For <i>in Vitro</i> Diagnostic Use		Caution!
	Lot Number		Version
	Expiration Date		Consult instructions for use
	Negative Control		Positive Control
	Contains reagents		Manufacturer

NAME

HPV High Risk Typing

INTENDED USE

HPV High Risk Typing is an *in vitro* nucleic acid amplification test for qualitative detection and genotyping of *Human Papillomavirus* (16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 66) in the urogenital swabs and biopsies.

PRINCIPLE OF ASSAY

HPV High Risk Typing Test is based on three major processes: sample preparation, multiplex amplification of DNA using specific *HPV* primers and detection of the amplified products on agarose gel. Each PCR-mix-1 tube contains primers directed against regions of four *HPV* types and β -globine gene used as Internal Control. If the swab is not correctly prepared (high quantity of mucous or insufficient quantity of epithelial cells) the Internal Control will not be detected.

MATERIALS PROVIDED

- **PCR-mix-1 "16-35"** (primers directed against regions of *HPV 16,31,33,35* and Internal Control (β -globine gene), 0,275 mL
- **PCR-mix-1 "18-59"** (primers directed against regions of *HPV 18,39,45,59* and Internal Control (β -globine gene), 0,275 mL
- **PCR-mix-1 "52-66"** (primers directed against regions of *HPV 52,56,58,66* and Internal Control (β -globine gene), 0,275 mL
- **2,5 x buffer-** 3 x 0,6 mL
- **TaqF Polymerase**, 0,09 mL
- **Mineral Oil**, 8,0 ml
- **Negative Control C-*** 1,6 ml;
- **DNA-buffer (C-)**, 0,5 mL
- **Internal Control** (β -globine gene), 0,2 mL
- **HPV Genotype Controls Panel** (types 16, 31, 33, 35; 18, 45, 39, 59; 52, 56, 58, 66), 12 x 0,15 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 μ l; 5-40 μ l) 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. Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
2. Do not pipette by mouth.
3. Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
4. Do not use a kit after its expiration date.
5. Dispose of all specimens and unused reagents in accordance with local regulations.
6. Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
7. Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
8. 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.
9. Material Safety Data Sheets (MSDS) are available on request.
10. 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.
11. Use of this product should be limited to personnel trained in the techniques of amplification.
12. 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 High Risk Typing must be stored at 2-8°C.

STABILITY

HPV High Risk Typing is stable up to the expiration date indicated on the kit label.

SAMPLE COLLECTION, STORAGE AND TRANSPORT

HPV High Risk Typing 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 freeze 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 for samples and controls (blue for PCR-mix-1 “16-35”, rose for PCR-mix-1 “18-59” and green for PCR-mix-1 “52-66”).
2. Prepare for each PCR-mix-1 one new tube and add for each sample 5*(N+1) µl of PCR-mix-1, 10*(N+1) of 2,5 x buffer and 0,5*(N+1) of TaqF Polymerase.
3. Add 15 µl of Reaction Mix into each sample tube. Add 1 drop (25 µL = of Mineral Oil).
4. Add to appropriate tube 10 µL of DNA sample obtained after sample preparation.
5. Prepare Controls as follows:
 - Negative Control: add 10 µL of DNA-buffer to the tube labeled *Cneg ampli*.
 - Internal Control: add 10 µL of Internal Control to the tube labeled *Cint*.
 - Positive controls:
 - of PCR-mix-1 “16-35”: add 10 µL of HPV 16, 31, 33 and 35 DNA (C+) to the 4 labeled blue tubes;
 - of PCR-mix-1 “18-59” add 10 µL of HPV 18, 39, 45 and 59 DNA (C+) to the 4 labeled rose tubes;
 - of PCR-mix-1 “52-66” add 10 µL of HPV 52, 56, 58 and 66 DNA (C+) to the 4 appropriate green tubes;
6. Close tubes and transfer them into the thermocycler only when temperature reaches 95°C and start the following program:

Thermocyclers with block temperature adjustment: “PTC-100”(MJ Research) BioRad, Biometra				Thermocyclers with active temperature adjustment: “PE 2400” (Perkin Elmer), Omn-E (Hibaid) and other.		
Step	t°C	Time	Cycles	t°C	Time	Cycles
1	95°C	Pause		95°C	Pause	
2	95°C	15 min	1	95°C	15 min	1
3	95°C	30 sec	42	95°C	30 sec	42
	63°C	40 sec		63°C	30 sec	
	72°C	50 sec		72°C	40 sec	
4	72°C	1 min	1	72°C	1 min	1
5	10°C	Storage		10°C	Storage	

RESULTS ANALYSIS

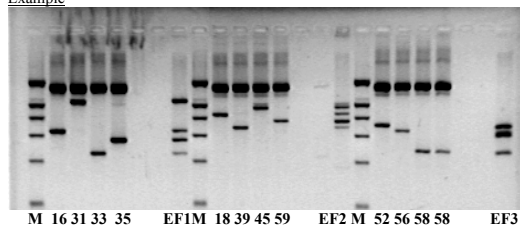
Analysis of PCR results is based on the presence or absence of specific bands of amplified DNA in Agarose gel (3%). Mix in the new tube the contents of the 4 tubes with amplified DNA of the 4 Controls (HPV 16, 31, 33 and 35). Repeat the same procedure for the controls of PCR-mix-1 “18-59” and PCR-mix-1 “52-66”. Add 10-15 µL of amplified products on the agarose gel. The length of specific amplified DNA fragments is:

PCR-mix-1 «16 – 35»		PCR-mix-1 «18 – 59»		PCR-mix-1 «52 – 66»	
Type	Lenght	Type	Lenght	Type	Lenght
HPV 16	325 bp	HPV 18	425 bp	HPV 52	360 bp
HPV 31	520 bp	HPV 39	340 bp	HPV 56	325 bp
HPV 33	227 bp	HPV 45	475 bp	HPV 58	240 bp
HPV 35	280 bp	HPV 59	395 bp	HPV 66	304 bp

Table 2. Results for controls

Control	Which step of test is controlled	Band 325 bp	Band 425 bp	Band 240 bp	Band 723 bp
<i>Cneg</i>	DNA isolation	No	No	No	No
DNA-buffer	Amplification	No	No	No	No
Internal Control	Amplification	No	No	No	Yes
HPV 16 DNA (+) C	Amplification	Yes	No	No	No
HPV 18 DNA (+) C	Amplification	No	Yes	No	No
HPV 58 DNA (+) C	Amplification	No	No	Yes	No

Example



M = marker

EF1 = 16, 31, 33, 35 Controls Amplification Mix

EF2 = 18, 39, 45, 59 Controls Amplification Mix

EF3 = 52, 56, 58 Controls Amplification Mix

RESULTS INTERPRETATION

- Detection of any band in the Negative controls (*Cneg* and DNA-buffer) indicates the contamination of kit reagents: the entire test protocol (sample preparation, amplification and detection) should be repeated. Discard any reagents that may be suspect.
- If the band of 723 bp (Internal Control) is absent, the negative result of this sample must be considered invalid. Absence of the band of the Internal Control indicates error of the specimens' collection (insufficient quantity of the epithelial cells in the swab).

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.

Analytical sensitivity: The kit HPV High Risk Typing allows to detect HPV DNA in 100% of the tests with a sensitivity of not less than 1000 copies/ml.

Target region: E6, E2, E1, L1 genes



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