















For *in Vitro* Diagnostic Use



## HPV High Risk Screen

### 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 Screen

### INTENDED USE

HPV High Risk Screen is an *in vitro* nucleic acid amplification test for qualitative detection of *Human Papillomavirus* (16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70) in the urogenital swabs and biopsies.

### PRINCIPLE OF ASSAY

HPV High Risk Screen 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. PCR-mix-1 tube contains primers directed against regions of *HPV* A6, A7, A9 groups (HPV types 16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66, 70) and  $\beta$ -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, 0,275 mL
- 2,5 x buffer, 0,6 mL
- Tag-F Polymerase, 0,03 mL
- Mineral Oil, 2,0 ml
- Negative Control\*, 1,2 ml;
- DNA-buffer (C-), 0,5 mL
- Internal Control ( $\beta$ -globine gene), 0,2 mL
- HPV C+ Positive Control (types 31, 39, 56), 1 x 0,2 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  $\mu$ l; 5-40  $\mu$ 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

-  Some components of this kit contain Sodium Azide as a preservative. Do not use metal tubing for reagent transfer.
- Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents. Thoroughly wash hands afterward.
- Do not pipette by mouth.
- 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.
- Biosafety Level 2 should be used for materials that contain or are suspected of containing infectious agents.
- Clean and disinfect all spills of specimens or reagents using a disinfectant such as 0,5% sodium hypochlorite, or other suitable disinfectant.
- 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.
- Material Safety Data Sheets (MSDS) are available on request.
- 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.
- Use of this product should be limited to personnel trained in the techniques of amplification.
- 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 Screen must be stored at 2-8°C.

## STABILITY

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

## SAMPLE COLLECTION, STORAGE AND TRANSPORT

HPV High Risk Screen 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

- Prepare required quantity of tubes for samples and controls.
- Add for each sample in the new sterile tube 5\*N µl of PCR-mix-1, 10\*N of 2,5 x buffer and 0,5\*N of Tag-F Polymerase.
- Add 15 µl of Reaction Mix into each sample tube. Add 1 drop (25 µL = of Mineral Oil).
- Add to appropriate tube 10 µL of DNA sample obtained after sample preparation.
- 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: add 10 µL of HPV C+ to the tube labeled *Cpos*.
- 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	40 sec		72°C	30 sec	
4	72°C	1 min	1	72°C	1 min	1
5	10°C	Storage		10°C	Storage	

## RESULTS ANALYSIS

Analysis of 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 – 267-325 bp
- Internal Control – 723 bp

## 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).

