PZP Human papillomavirus Qualitative PCR

Version 6

The kit is designed for professional use in specialized clinical and research laboratories.

Contents of the Kit :

Kit composition	(25 reactions)
HPV Mix	380 µl
Positive control (10 copies/ µl)	1 x 50 µl

Technical Specification :

Technology: Conventional PCR

Target sequence: L1 gene from HPV genome.

Specificity: > 40 HPV genotypes.

High Risk: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52,53,55, 56, 58, 59, 66, 67, 68, 70, 73, 82.

Low Risk: 6,7,11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 85 , 89,90,91,114.

Sensitivity: ≥ 25 HPV copies/ 2000 cells or 50 ng DNA with the probability of 95%.

Storage: -20 °C

Storage of the Kit :

All reagents of the kit should be stored at -20°C. They are stable till expiry at this temperature. Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of assay. If the kit is to be used only occasionally, the reagents should be frozen in aliquots.

Description :

This kit is designed for the detection of the human papillomavirus genomic DNA based on the detection of the L1 consensus region, using the Polymerase Chain Reaction (PCR) and the detection of the resulting amplification products by agarose gel electrophoresis. HPVs are classified into low and high-risk categories based on their association with malignant lesions. The low-risk types mostly cause the development of genital condylomata (warts). The high-risk types HPV induce cervical squamous intraepithelial lesions (SIL), which in turn are classified in low (LSIL) and high grade (HSIL) in severity, and which may progress to cervical cancer.

Sampling and sample storage :

Samples of Vaginal secretions and other body fluids must be inserted into a proper fixator (Ethanol-Acetic acid). Store and transport at the temperature of 4°C within 24 hours. If longer storage period is required, freeze to -20°C.

DNA isolation :

Isolation recommended by means of commercial DNA isolation kits according to the particular protocols of the isolation kit manufacturers. We recommends the following isolation kits: High Pure Viral Nucleic acid Kit (Roche), PZP Nucleic Acid Template Extraction Kit (Iran) and others proper Kits.

PCR Amplification :

1. Add **15µl** of the HPV Mix to all of tubes and then add **10 µl** of extracted DNA, Positive control, and distilled water to sample tubes, Positive control tube and negative control tube, respectively. The final reaction mix volume should be **25 µl**.

2. Insert the tubes into a Thermal cycler and amplify them according to the following recommended program:

PCR program (Touchdown PCR):

Stages	Repeat Number
95 °C/15 min	**1**
95 °C/35 sec **63°C/15 sec** **72 °C/35 sec**	**12**
95 °C/35 sec **61 °C/15 sec** **72 °C/35 sec**	**12**
95 °C/35 sec **58°C/15 sec** **72 °C/35 sec**	**20**
72 °C/5 min	**1**

Agarose Electrophoresis :

The resulting amplification products should be separated on 1% agarose gel electrophoresis (5V/cm). DNA staining performed to GelRed Nucleic Acid Gel Stain (10X) (PZP, Iran) and visualized by means of UV illumination. Since the loading buffer is included in the reaction mix, there should be directly loaded at least **5 µl** of the amplification product.

Detection Evaluation :

Positive Control is the detected amplification product with the length of **450 bp**. Negative Control not detected any the amplification product in negative tube. Sample Tube: The presence of **450 bp** fragments indicates positive test. If the positive control does not amplify, reaction should be repeated.