F-HPV typing™ Kit

Quick PCR set up Protocol

Thaw PCR Mix and PRIMERS Mix ***F*-HPV typing™** vials and mix thoroughly by vortexing a few seconds.

PCR mix and PRIMERS must be mixed freshly before PCR.

Prepare PCR and PRIMERS Mix according to the table below for sample.

PCR Mix	12,5 µL
PRIMERS Mix	7,5 µL
Mixture PCR Final Volume	**20,0 µL**

Aliquot 20 µL of the Mixture PCR in each PCR tube and add 5 µL of extracted DNA.

<u>Warning</u>: In order to avoid possible contamination, *F*-HPV typing™ mix must be aliquoted in the PCR area with dedicated pipettes and filtered tips. One drop of mineral oil on each PCR tube will also reduce the risk of contamination by amplicons generated in the previous PCR.

Performing PCR

In order to increase the PCR specificity, Hot Start Taq Polymerase is included in the reaction buffer. The enzyme is totally inactive at room temperature. This allows easy set-up of PCR reaction without ice. Activation is achieved with 15 min. hold at 95 °C.

1. Program the Thermalcycler according to the following parameters:

Taq Activation	Denaturation	Annealing	Extension	Final extension	Storage
Hold	**35 Cycles**			**Hold**	**Hold**
95 °C 15 min	95 °C 30 sec	64 °C 30 sec	72 °C 30 sec	60 °C 10 min	4-20 °C ∞

2. Place tubes in Thermalcycler and close the lid
3. Start the PCR
4. PCR products are stable at room temperature overnight, longer storage before electrophoresis should be at 4 °C.

<u>Warning</u>: After PCR is completed, tubes should never be opened in the PCR set-up area. This is essential in order to avoid contamination at any future PCR amplification.