MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Detection Kit for Human Papilloma Virus 16/18 DNA

Product Number: QDHPV16/18

Shipping and Storage

1. Store below 30°C. It is valid for 12 months.
2. Date of manufacture and term of validity: see the label.
3. Transport at normal temperature, not suggested over 14 days.
4. Opened but not completely used HPV 16/18 PCR Master Mix should be stored at (-20±5) °C. It is recommended to separate in PCR tubes before refrigeration to avoid repeated freezing and thawing of all reagents next time. Storage time should not exceed 21 days.

Components

Reagent	QDHPV16/18 48T	Ingredients
HPV 16/18 PCR Master Mix	Lyophilized powder	Contains target gene primers, probes, reaction buffer, dNTPs, Mg^{2+}, Taq Enzyme
Redissolved Diluent	1.40ml	Purified water
HPV 16/18 Positive Control	Lyophilized powder	Recombinant plasmid containing target gene
HPV 16/18 Negative Contro	0.25ml	Physiological saline

Note: Do not mix reagents from different batches.

Description

This kit uses real-time fluorescence PCR detection technology to achieve qualitative detection of HPV type 16 and type 18 in genitourinary tract secretion samples by using a pair of specific primers and a specific fluorescent probe in the conserved region shared by human papillomavirus (HPV) type 16 and type 18.

This kit sets internal control, to monitor the presence of PCR inhibitors in the samples by detecting whether the internal control is normal, so as to avoid PCR false negative result.

Application

This kit is used to qualitatively detect the nucleic acid of Human Papilloma Virus16/18 in male urethral swab samples, female cervical swab samples in vitro.

The test results can be used for auxiliary diagnosis of sexual transmission and reproductive tract infectious diseases caused by human papilloma virus type 16and type 18. The test results are only for clinical reference and should not be used as the sole criterion for clinical diagnosis.

Material Required but Not Provided

Amplification Instrument: ABI7500, ABI 7500 Fast and other Real-time fluorescence PCR instrument with FAM and VIC channels.

Vortex shaker, centrifuge, pipette sets with tips (10μL, 20μL, 1000μL), PCR tubes, clean bench and Bio Safety Cabinet (BSC), disposable gloves, shoe covers, hats, eyes shields and personal protective equipment (PPE).

Sample Requirements

1. Applicable sample types: male urethral swab samples, female cervical swab samples.
2. **Sample collection:**

2.1. Male: Take urethral secretions or a small cotton swab into the urethra for 2 to 4 cm, and twist the swab to remove the

secretions.

2.2. Women: Wash the exocervical secretions with a sterile saline cotton ball, then insert a sterile cotton swab into the cervix, and then rotate the cotton swab to collect cervical secretions after stopping for a few seconds.

Put the sampled cotton swab into about 1mL of normal saline to wash thoroughly, squeeze dry against the wall, and discard the cotton swab.

3. Sample storage and transportation:

Samples to be tested within 72 hours should be stored at 2-8℃. Samples to be tested within 90 days should be stored at -20℃. The specimens should be transported by sealed ice jug with ice or foam box with ice.

Protocol

1. Reagent preparation

Take out the HPV 16/18 PCR Master Mix, open the bottle cap according to the arrow direction of the aluminum-plastic cover, add 1mL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 to 60 seconds until the liquid is clear and transparent. Subpackage it into PCR reaction tubes according to 20μL/ tube.

2. Nucleic acid extraction

This kit is not included for Nucleic Acid (NA) extraction reagent.Commercially available extraction kits that have been shown to generate highly purified DNA when following manufacturer's recommended procedures for sample extraction are applicable.

If the extracted DNA is not used immediately, it should be stored below -20℃.

For long-term storage, it should be stored below -80℃.

Note: Sample to be tested and HPV 16/18 Negative Control need to be extracted simultaneously, HPV 16/18 Positive Control does not require extraction, but needs to be redissolved with 50μL Redissolved Diluent and mixed well before use.

3. Sample adding

Add the corresponding solution to the PCR reaction tubes according to the:

Sample Type	Operation Instruction
Sample under test	Add 5μL extracted nucleic acid from step 2 to the reaction tube, and cover the cap
Negative/Positive control	Add 5μL of the Negative control extracts prepared in step 2 and 5μL of the Positive control to each reaction tube, and close the tubes' cap tightly

The total reaction volume is 25μL.

After adding the sample, the PCR reaction tubes should be centrifuged for 15s on a palm centrifuge and then delivery to the nucleic acid amplification region.

If bubbles are found, the tube wall should be gently flicked to remove bubbles and centrifuged again.

4. PCR amplification

4.1. Place the reaction tube in the automatic fluorescent PCR machine, set the parameters of negative control, positive control and sample to be tested for PCR reaction with reference to the instrument operation instructions, and record the corresponding sample name.

4.2. Select FAM channel to detect HPV 16/18 nucleic acid. Select VIC channel to detect internal control. Set the Reaction Volume per Well to 25μL.

(Note: For ABI series instruments, select 'None' under 'Quencher',and select 'None' as the dye to use as the passive reference.)

4.3. Recommended setting of reaction procedure:

Step	Temperature (℃)	Time	Cycle
Pre-denaturation	95	2 min	1
Denaturation	95	5 s	40
Annealing, extension and fluorescence collection	60	35 s	

5. Results analysis

After the reaction is completed, the results are automatically saved.

The Start value, End value and Threshold value of the Baseline should be adjusted according to the analyzed image (the user can adjust it according to the actual situation, the Start value can be set at 3~15, the End value can be set at 5~20, the amplification curve of the Negative control should be adjusted to be flat or below the threshold line).

Click Analyze for analysis, make the parameters meet the requirements in the following '6. Quality control', and then go to the Plate window to record the Ct value.

6. Quality Control

6.1. Negative control: No significant amplification curves for FAM and VIC detection channels.

6.2. Positive control: FAM and VIC detection channels have obvious amplification curves and Ct value ≤ 35.00.

The above requirements must be met at the same time in the same experiment, otherwise this experiment is invalid and needs to be repeated.

Positive judgment value

Through the study of the reference value, it was determined that the Ct positive judgment value of FAM and VIC channels both were 35.00.

Explanation of Test Result

1. FAM channel:

   1.1. Ct value ≤ 35.00 and the amplification curve is typical S-shape: HPV 16/18positive.

   1.2. Ct value > 35.00 or no Ct value: HPV 16/18 negative.

2. VIC channel:

   For samples with positive FAM channel, there is no requirement for internal control test results; For samples with negative FAM channel, the internal control should be positive (Ct value ≤35), if the internal control Ct value >35 or no display, the test result of the sample is invalid, the reason should be found and eliminated, and the sample should be re-sampled for repeated experiment.

Limitation

1. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests and treatment response.
2. The sample test results are related to the quality of sample collection, processing, transportation and storage, any mistakes made during these periods may result in inaccurate results. False-positive results may occur if cross-contamination is not controlled during sample processing.
3. The detection of this kit is for the conserved region of the pathogen, false-negative results may occur because the pathogen mutated genetically during an epidemic.
4. This kit is limited to the sample types and applicable models specified in this manual. Validation should be performed before using other sample types and models.
5. A negative test result only means that the viral load in the specimen is lower than the detection limit of this kit, but does not rule out the possibility of infection with HPV 16/18.

Performance Parameters

1. Limit of Detection: The minimum detection limit of this reagent is 1.00×10^3copies/mL.
2. Precision: The same batch and different batches precision was consistent with the coefficient of variation (CV, %) of the Ct value of the test results ≤5%.
3. Compliance rate of negative/positive reference products: The compliance rate of negative reference products in enterprise reference is 100%, and the compliance rate of positive reference products is 100%.
4. Analysis specificity

4.1. Cross-reaction: There is no cross-reaction with other types of HPV (such as HPV 6, HPV 11, etc.), Neisseria gonorrhoeae, Chlamydia trachomatis, Ureaplasma urealyticum, adenovirus, cytomegalovirus, Epstein-Barr virus, herpes simplex virus types 1 and 2, Staphylococcus aureus

4.2. Interfering substances: Mucin, blood, Jieeryin lotion, miconazole nitrate, etc., respectively do not interfere with the test results of the kit.

Note

1. This kit is used for in vitro auxiliary diagnosis only, please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps.
2. Before the test, please be familiar with and master the operation method and precautions of various instruments to be used, and carry out quality control for each experiment.
3. The entire experimental operation process and the hardware and software facilities of the PCR laboratory should comply with the requirements of the Ministry of Health. The operator must be trained in the use of real-time fluorescent PCR instruments.
4. The experimental process should be carried out in different areas (reagent preparation area, sample preparation area, amplification and product analysis area). Special instruments and equipment should be used in each stage of the experimental operation. There should be strict requirements on the flow of people and air in each section to avoid cross-contamination to the maximum. Laboratory consumables (e.g. centrifuge tubes, suction tubes) should have proper cleaning and quality control procedures to avoid RNA enzyme contamination or false negative results that caused by amplification reaction inhibitors.
5. In order to avoid any potential biological hazards from the sample, all test samples should be regarded as an infectious substance. Wear overalls, disposable masks and gloves during the experiment, and replace gloves frequently to avoid cross-contamination between samples. The sample operation and processing must meet the requirements of relevant regulations.
6. Samples to be tested must be fully thawed and mixed at room temperature before use.