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Diagnostic Kit for High-risk Human Papillomavirus (HPV) DNA(Type 16, 18、31、33、35、39、45、51、52、56、58、59、66、68)

Product Number: DTL0435

Shipping and Storage

1. Store below 30°C. It is valid for 12 months.
2. Transport at normal temperature, not suggested over 14 days.
3. Opened but not completely used the all components 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. It is not recommended to repeat the freeze-thaw cycle more than 7 times.
4. Date of manufacture and term of validity: see the label.

Component

Component	48T
HPV PCR Master Mix 1	Lyophilized powder ×1 Bottle
HPV PCR Master Mix 2	Lyophilized powder ×1 Bottle
HPV PCR Master Mix 3	Lyophilized powder ×1 Bottle
HPV PCR Master Mix 4	Lyophilized powder ×1 Bottle
Positive Control	100μL
Negative Control	1mL
Redissolved Diluent	1.5mL

1. Do not mix reagents from different batches.
2. The reaction system is lyophilized powder that contains all components required for fluorescence PCR, including Taq enzyme, reverse transcriptase, primers, probes, dNTPs, and Mg^{2+}.

Description

This kit uses real-time fluorescence PCR detection technology to achieve qualitative detection of HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 in genitourinary tract secretion samples by using a pair of specific primers and a specific fluorescent probe in the conserved region shared by HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68.The kit is provided with an Internal Control（IC）, which can monitor whether there is PCR inhibitor in the sample to be tested by detecting whether the internal control is normal or not, so as to avoid false negative PCR.

The kit contains 4 bottles. The specific test items are as follows:

Contents	Test item	Detection Channel	Contents	Test item	Detection Channel
HPV PCR Master Mix 1	HPV 16	FAM	HPV PCR Master Mix 3	HPV 33	FAM
	HPV 31	VIC（HEX）		HPV 35	VIC（HEX）
	HPV 56	ROX（TEXAS RED）		HPV 68	ROX（TEXAS RED）
	HPV 52	CY5		HPV 66	CY5
HPV PCR Master Mix 2	HPV 18	FAM	HPV PCR Master Mix 4	HPV 39	FAM
	HPV 45	VIC（HEX）		HPV 51	VIC（HEX）
	HPV 59	ROX（TEXAS RED）		Internal Control(IC)	ROX（TEXAS RED）
	HPV 58	CY5			

Application

This kit is suitable for qualitative detection of 14 high-risk HPV DNA (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in male urethral swab samples and female cervical swab samples in vitro.

Cervical cancer (CC) is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death in women worldwide. It has been demonstrated that persistent infection with various human papillomavirus (HPV) genotypes plays a major role in the development of high and low-grade cervical intraepithelial neoplasia (CIN) and CC. Human papilloma virus (HPV) is a small, double-stranded, circular DNA virus. HPV infection is considered the most common viral sexually transmitted infection worldwide. HPV has been classified genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 as carcinogenic to humans. The test results can be used for auxiliary diagnosis of sexual transmission and reproductive tract infectious diseases caused by human papillomavirus genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. The test results are only for clinical reference and should not be used as the sole criterion for clinical diagnosis.

Applicable instruments

Real-time fluorescence PCR instrument with FAM, VIC(HEX), ROX(TEXAS RED), CY5 channels.

Specimen collection

1. Applicable sample type: Male urethral swab samples, female cervical swab samples.
2. Sample collection:
   - 2.1. Male samples: Take urethral secretions or a small cotton swab into the urethra for 2 to 4cm, and twist the swab to remove the secretions.
   - 2.2. Female sample: 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: The collection or processing sample should not exceed 24 hours under the conditions of 2℃ ~ 8℃. If long-term preservation is needed, it should be stored below -70°C, and the freezing fusion should not exceed 3 times.

Protocol

1. Reagent preparation:

Take out the HPV PCR Master Mix 1-4, open each bottle cap according to the arrow direction of the aluminum-plastic cover, add 960μL of Redissolved Diluent, strongly mixed on the vortex for more than 1 minute, then stand for 30 ~ 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℃ and avoid repeated freezing and thawing.

Note:The Negative Control and the Positive Control does not require nucleic acid extraction.

3. Add sample:

The correspond substances were added to that above PCR reaction tubes according to the following table:

Type	Add sample description
Testing Sample	Add 5μL of the extract prepared in step 2 to the reaction tube(One sample add separately to four reaction hole), and close the tube cover.
Negative Control/ Positive Control	Add 5μL of negative control and positive control to the reaction tube, and cover the tube 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. RT-PCR amplification:

Place the reaction tube in the automatic fluorescent PCR instrument, set the Negative control, Positive control, and test sample parameters to perform PCR experiment according to the operating instructions of the instrument, and record the corresponding sample name.

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

The kit contains 4 reaction system. Select the amplification detection channels.The specific test items and detection channels are as follows:

Contents	Test item	Detection Channel	Contents	Test item	Detection Channel
HPV PCR Master Mix 1	HPV 16	FAM	HPV PCR Master Mix 3	HPV 33	FAM
	HPV 31	VIC（HEX）		HPV 35	VIC（HEX）
	HPV 56	ROX（TEXAS RED）		HPV 68	ROX（TEXAS RED）
	HPV 52	CY5		HPV 66	CY5
HPV PCR Master Mix 2	HPV 18	FAM	HPV PCR Master Mix 4	HPV 39	FAM
	HPV 45	VIC（HEX）		HPV 51	VIC（HEX）
	HPV 59	ROX（TEXAS RED）		Internal Control(IC)	ROX（TEXAS RED）
	HPV 58	CY5			

Set the Reaction Volume per Well to 25μL. Recommended reaction program setting:

Step	Cycles	Temperature	Time	Collect fluorescence signal
1	1 cycle	95℃	2min	No
2	45 cycles	95℃	15sec	No
		60℃	30sec	Yes

5. Result 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

Negative Control: FAM,VIC (HEX) ,ROX (TEXAS RED) ,CY5 detection channel has no obvious amplification curve. Positive Control: FAM,VIC（HEX）,ROX（TEXAS RED）,CY5 detection channel all has an obvious amplification curve, and the 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, the minimum detection limit of this kit for HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58,59, 66, 68 was determined to be 500 copies/mL, and the Ct positive judgment value was 35.00 and the Ct positive judgment value of IC was 35.00.

Contents	Detection Channel	Negative Control	Positive Control	Contents	Detection Channel	Negative Control	Positive Control

HPV PCR Master Mix 1	FAM	No CT value	CT value≤35.00	HPV PCR Master Mix 3	FAM	No CT value	CT value≤35.00
	VIC	No CT value	CT value≤35.00		VIC	No CT value	CT value≤35.00
	ROX	No CT value	CT value≤35.00		ROX	No CT value	CT value≤35.00
	CY5	No CT value	CT value≤35.00		CY5	No CT value	CT value≤35.00
HPV PCR Master Mix 2	FAM	No CT value	CT value≤35.00	HPV PCR Master Mix 4	FAM	No CT value	CT value≤35.00
	VIC	No CT value	CT value≤35.00		VIC	No CT value	CT value≤35.00
	ROX	No CT value	CT value≤35.00		ROX	No CT value	CT value≤35.00
	CY5	No CT value	CT value≤35.00				

Explanation of Test Result

When quality control conditions are met, the amplification curve of the sample to be tested is a typical S-shaped curve, and the Ct value is ≤35.00 and meets the following conditions, it will be judged as positive.

If the Ct value of the test sample is >35.00 and the Ct value of the Internal Control (ROX/TEXAS RED Channel) is ≤35.00, it will be judged as negative.

If the Ct value of the test sample is >35.00 and the Ct value of the Internal Control (ROX/TEXAS RED Channel) is >35.00, the test result of the sample is invalid and the cause should be found and eliminated. Samples need to be recollected and the experiment repeated.

Correspondence of results with HPV types is shown in the table:

Contents	FAM Channel	VIC (HEX) Channel	ROX(TEXAS RED) Channel	CY5 Channel
HPV PCR Master Mix 1	Type 16	Type 31	Type 56	Type 52
HPV PCR Master Mix 2	Type 18	Type 45	Type 59	Type 58
HPV PCR Master Mix 3	Type 33	Type 35	Type 68	Type 58
HPV PCR Master Mix 4	Type 39	Type 51	Internal Control	/

Limitation

1. Sample detection results are related to sample collection, processing, transportation and preservation quality.
2. There is no control of cross -pollution during the sample extraction process, and false positive results will occur.
3. Positive control and leakage of amplification products can lead to false positive results.
4. The genetic mutations and reorganizations during epidemics can lead to false negative results.
5. Different extraction methods have differences in extraction efficiency, which will lead to false negative results.
6. Reagent transportation, improper preservation, or inaccurate reagent preparation reagent detection performance decreases, and the results of false negative or quantitative detection occur.
7. The results of this test are for reference only. If the diagnosis must be confirmed, please combine clinical symptoms and other test methods.

Performance Parameters

1. Limit of Detection: The minimum detection limit of this reagent for HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58,59,66 and 68 is 500 copies/mL.

2. Precision: Repeat detection of the enterprise precision reference product 10 times, and the coefficient of variation (CV, %) value of detected concentration logarithm is ≤5.00%.
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 of Specificity:
   4.1. Cross-reaction: There is no cross-reaction with other types of HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58,59,66 and 68, 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, Jie er yin lotion, miconazole nitrate, etc., respectively do not interfere with the test results of the kit..

Note

1. 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 reaction solution should be stored away from light.
4. Try to avoid bubbles in the reaction, and the tube cover needs to be tight.
5. Use disposable heads, disposable gloves and special work clothes in each district.
6. Sample processing, reagent preparation, and samples need to be performed in different areas to avoid cross -pollution.
7. After the experiment is completed, use 10% hypochloride or 75% alcohol or ultraviolet light to treat the workbench and pipette.
8. All items in the kit should be treated as pollutants and processed in accordance with the "Biological Safety General of Microbiological Biomedical Laboratory".