Human Papillomavirus (28 types) Nucleic Acid Test Kit (Fluorescence PCR) Instruction for Use (V1.0)

[REF] HWTS-CC009A

[Specification] 50 tests/kit

[Research Use Only]

This kit is used for qualitative detection of 28 types of human papillomavirus (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 73, 81, 82, 83) in female cervical exfoliated cells, but is not used for completely genotyping.

Cervical cancer is one of the most common malignant tumors of female reproductive tract. Previous studies have shown that persistent infection and multiple infection of human papillomavirus is one of the important causes of cervical cancer. To establish a simple, specific and rapid etiological detection method is of great significance in cervical cancer.

[Test Principles]

The kit uses multiple nucleic acid amplification (PCR) fluorescence detection method. Highly specific primers and probes are designed based on the L1 gene target sequence of HPV. The specific probe is labeled with FAM fluorophore (HPV6, 11, 16, 18, 31, 54, 56, 83), VIC fluorophore (HPV26, 44, 61, 81 and internal control), CY5 fluorophore (HPV40, 42, 43, 45, 51, 52, 53, 82) or ROX fluorophore (HPV33, 39, 35, 58, 59, 66, 68, 73) at 5', and the 3' quencher group is BHQ1 or BHQ2. During the PCR amplification, specific primers and probes bind to their respective target sequences. When Taq enzyme encounters the probes bound to the target sequence, it exerts the function of 5' end exonuclease to separate the reporter fluorophore from the quencher fluorophore, so that the fluorescence monitoring system can receive the fluorescent signal, that is, every time a DNA strand is amplified, a fluorescent molecule is formed, which realizes the complete synchronization of the accumulation of fluorescent signals and the formation of PCR products, so as to achieve qualitative detection of 28 types of human papillomavirus (HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 73, 81, 82, 83) in cervical exfoliated cell samples.

[Kit Contents]

No.	Name	Specification	Description
1	HPV Reaction Buffer 1	1.25mL/vial×1	It is prepared from HPV16,18, 31, 33, 35, 45, 51, 52, 53, 56, 58, 66 (high risk). and internal control specific primers and fluorescent probes in a certain proportion
2	HPV Reaction Buffer 2	1.25mL/vial×1	It is prepared from primers and fluorescent probes specific to HPV6, 11, 26, 39, 40, 42, 43, 44, 54, 59, 61, 68, 73, 81, 82, 83 (low risk) in a certain proportion.
3	HPV Enzyme Mix	55 μL/vial×1	A mixture of Taq enzyme, UNG and glycerol
4	HPV Positive Control 1	150 μL/vial×1	A mixture of high-risk templates and internal control template
5	HPV Positive Control 2	150 μL/vial×1	A mixture of low-risk templates
6	HPV Blank Control	300 μL/vial×1	RNase-free and DNase-free water

Noted: The contents in different batches of reagents are not interchangeable within the shelf life.

Reagents required but not provided:

Viral genomic DNA/RNA Extraction Kit produced by Ultrassay or other equivalent kits.

1.5 mL RNase-and DNase-free centrifuge tubes, RNase-free and DNase-free tips, 0.2 ml PCR reaction tube, desktop centrifuge, and desktop oscillation mixer.

[Storage Conditions and Shelf Life]

This kit should be stored below-18°C and protected from light. Its shelf life is 9 months. After opening, please use it up within 3 months. It shall not be subjected to repeated freezing and thawing for more than 4 cycles. It shalt be transported below-18°C and protected from light, and can be stably stored for 5 days.

[Applicable Equipment]

Applied Biosystems 7500 Real-Time PCR Systems, Ultrassay XP96 Real Time qPCR System

[Acceptable Specimens]

Before sampling, gently wipe the excessive secretions of the cervix with a cotton swab, replace the cotton swab, use a cotton swab infiltrated with cell preservation solution or a sampling brush for cervical exfoliated cells to close to the cervical mucosa, and turn clockwise for 3-5 cycles to obtain cervical exfoliated cells. Slowly take out the cotton swab or brush, and put into the sample tube with 1 ml of sterile saline. After rinsing thoroughly, squeeze the cotton swab or brush against the wall and discard. Tighten the cap, and mark the sample name (or number) and type on the sample tube.

2. Storage

The sample n be tested should not be stored at 2-8°C for more than 48 h, should be stored below-18℃ for no More than six months, and can be stored below-70°C for a long time. Avoid repeated freezing and thawing.

3. Transportation

It is recommended to adopt ultra-low temperature transportation with liquid nitrogen and dry ice.

[Test Procedures]

1. Reagent preparation (reagent preparation area)

1.1 Take out the components of the kit and place at room temperature. After the components are equilibrated to room temperature, shake and mix well, and centrifuge briefly for later use.

1.2 Take out the HPV reaction buffer from the kit, dissolve on ice, and shake and mix. Calculate the number (n) of samples to be prepared (n = number of samples + blank control + positive control). Prepare PCR-Mix according to the following table, add 25 μL of PCR-Mix to the PCR reaction tube, press the cap tightly and quickly transfer to the sample processing area. The operation should be performed on ice. Immediately store the remaining HPV reaction buffer below -18°C.

	HPV Reaction Buffer	HPV Enzyme Mix (uL)
PCR-Mix 1	24.45xn (HPV Reaction Buffer 1)	0.55xn
PCR-Mix 2	24.45xn (HPV Reaction Buffer 2)	0.55xn

2. Sample processing (sample processing area) (positive control and blank control do not need to be processed)

The extracted genomic DNA from a female cervical exfoliated cell sample should ensure quality and the amount of DNA required for the experiment, and should be stored below-18°C.It is recommended to use the viral genomic DNA/RNA extraction kit produced by Ultrassay. or other commercially available viral DNA extraction kits (such as: QIAamp DNA Mini Kit (QIAGEN) (Cat.No.51304)) to extract strictly according to the instructions.

3.Sample loading

Add 5 uL of each of DNA extracted in step 2, the blank control, and the positive control to the PCR-Mix aliquoted in step 1 on ice, tighten the cap, and centrifuged briefly. Put the PCR reaction tube into the fluorescent PCR detector and record the sequence of sample loading.

4.PCR amplification (detection area)

Setting of instrument detection channel:

PCR-Mix	Fluorophore	Quencher group
PCR-Mix 1/2	FAM	None
	CY5	None
	VIC (HEX)	None
	ROX	None

Reference Dye: Select Nome (only applicable to ABl series instruments);

PCR reaction conditions are set as shown in the table below, and reaction volume is set to 30 ul. For specific detection channel settings, refer to the instructions of use of each instrument

Step	No. of Cycle	Temperature	Duration	Collect Fluorescent Signal
1	1	50°C	2 minutes	No
2	1	95°C	10 minutes	No
3	10	95°C	15 seconds	No
		62°C	20 seconds	
4	30	95°C	15 seconds	No
		58°C	30 seconds	Yes

[Reference Range]

The ROC curve analysis method is used to analyze the critical value of the kit. The positive sample reference Ct value≤28, The Ct value of the internal control is≤25.

[Explanation of Test Results]

1. If the Ct value for detecting FAM/CY5/ROX with PCR-Mix Well 1≤28, It is judged as positive one or more of HPV16, 18, 31, 33, 35, 45, 51, 52, 53, 56, 58, 66; the specific types and channels are shown in the table below.
2. If the Ct value for detecting FAM/CY5/VIC (HEX)/ROX with PCR-Mix well 2≤28, It is judged as positive for one or more of HPV6, 11, 26, 39, 40, 42, 43, 44, 54, 59, 61, 68, 73, 81, 82, 83; The specific types and channels are shown in the table below:

Channel and Type Correspondence Table

No	Channel	Type
PCR-Mix 1	FAM	16, 18, 31, 56
	VIC (HEX)	Internal Control
	CY5	45, 51, 52, 53
	ROX	33, 35, 58, 66
PCR-Mix 2	FAM	6, 11, 54, 83
	VIC (HEX)	26, 44, 61, 81
	CY5	40, 42, 43, 82
	ROX	39, 59, 68, 73

If there is a possibility that the above two HPV reaction buffers may be positive at the same time, it is reported as positive for HPV.

3. If the Ct value for detecting FAM/CY5/VIC (HEX)/ROX channel with PCR-Mix well 1 is 0 or >28, the Ct value for detecting FAM/CY5/VIC (HEX)/ROX channel with PCR-Mix well 2 is 0 or >28, and Ct value of the internal control is ≤25, it is reported as negative.
4. If the Ct value in the internal control >25, then a retest is required to be performed.

[Limitations of Test Method]

1. The test results obtained by this kit are for laboratory reference only. The final conclusion should be conducted in combination with their symptoms/signs, medical history and other laboratory tests.
2. Unreasonable sample collection, transportation, storage and processing may lead to incorrect test results.
3. The contamination of amplification products and cross-contamination between samples in nucleic acid extraction are prone to false positive results. Therefore, the laboratory should strictly follow the Working Specifications for Gene Amplification Laboratories for the equipment and operators, and the operation should be carried out in strict accordance with the instructions.
4. A negative result does not mean that the patient is not infected with HPV, and the conclusion must be combined with other results. The possible causes of a negative result include: (1) Unreasonable sample collection, transportation and processing, low pathogen titers in the sample;(2) Variation of the pathogen detection target sequence; (3) Other interference factors that have not been verified.

[Product Performance]

1.The kit is intact in appearance, and the extractable volume of each vial is correct.

2.The Ct value for detecting each channel of blank control should be 0 or of no value, and the Ct value for detecting each channel of positive control should be ⩽30.

3.Specificity: Each nucleic acid reaction solution detects HPV negative control, and the test results should all be negative.

4.Accuracy: Each nucleic acid reaction solution is used to detect the corresponding in-house positive reference standard, and the test results should all be positive for the corresponding type.

5.Limit of Detection: the LoD of each reaction buffer should not exceed 50 copies/reaction.

6.Repeatability: Each reaction buffer is used to detect the repeatability reference, the test is repeated in 10 wells, and the coefficient of variation (CV%) of Ct value should not be more than 5.0%.

[Precautions]

1. The results obtained by this kit may be affected by the source of the sample itself, sample collection process, sample quality, sample transportation conditions, sample pretreetment and other factors, and limited by the quality of extracted DNA, working status of fluorescent quantitative PCR system, operating environment, and limitations of current molecular biology, resulting in false positive or false negative test results. Users must understand the potential errors and accuracy limitations that can arise during testing.
2. The kit should be transported and stored at a low temperature. Before use, the reagents in the kit should be fully thawed and shaken evenly, and centrifuged briefly. Unnecessary repeated thawing and freezing of the reagents in the kit should be avoided.
3. All reagents in this kit are specially prepared for the detection of the above mutation sites. Replacement of any reagent in the kit may affect its effect. The components of kits in different batches cannot be mixed with each other.
4. When carrying out a test, perform different operations strictly according to different areas: Area 1: pre-PCR preparation area - prepare the reagents for amplification;

Area 2:sample processing area-process the samples to be tested and reference standards;

Area 3: detection area - PCR amplification and detection.

The relevant laboratory management specifications are strictly implemented in accordance with the management regulations of the gene amplification testing laboratory issued by the administrative department.

Items in each area are for exclusive use and must not be cross-used to avoid contamination; please clean the workbench immediately after testing. The lysates of samples stored below -18°C or -70℃ should be thawed at room temperature before sample loading and used after centrifugation for a while.

5. During testing, prevent the contamination of reagents by exogenous DNA, and add the sample DNA, and finally add the positive control. It is recommended to use separate, dedicated pipette and tips when preparing reaction reagents and adding a DNA template.

6. The reaction tube aliquoted with the reaction solution should be capped or put into an airtight bag and then transferred to the sample processing area.

7. When the reaction solution is aliquoted, try to avoid generating bubbles. It is necessary to check whether the reaction tubes are tightly capped before loading to prevent the leakage of fluorescent substances from contaminating the instrument.

8. When added, the sample should be completely dropped into the reaction solution, and should not stick to the tube wall, and the tube cap should be tightly closed as soon as possible after sample adding.

9. Take out the reaction tube immediately after amplification, seal in a dedicated plastic bag, and discard in the designated place.

10. The centrifuge tube and tips used during testing must be free of RNase and DNase. The centrifuge tube and tips used during testing must be processed in a harmless manner. The used tips should be directly thrown into the waste tank containing 84 disinfectant, and sterilized together with other waste products before discarding.

11. The workbench and various laboratory supplies should be regularly disinfected with 75% alcohol or ultraviolet light.

12. All chemicals are potentially dangerous. During operation, please wear appropriate laboratory overalls, wear disposable gloves, and take protective measures. The used kits are laboratory wastes and should be disposed of properly.

[References]

1. Li Hua,Gao Guo-lan. Research progress of HPV and cervical cancer[J]. Practical Journal of Cancer, 2007,22:420-422.

2. He Guishan.Research progress in detection of human papillomavirus[J]. Gansu Medicine, 2009,28:182-183.

3. Ning Tang, Shihai Huang, Brian Erickson, etc. High-risk HPV detection and concurrent HPV 16 and 18 typing with abbott realtime high risk HPV test[J]. Journal of Clinical Virology, 2009, 45:S25-S28.

[Index of Symbols]

Symbols	Meanings	Symbols	Meanings
RUO	RESERCH USE ONLY	REF	CATALOGUE NUMBER
	MANUFACTURER		CAUTION
	USE BY DATE		TEMPERATURE LIMITATION
LOT	BATCH CODE		CONSULT INSTRUCTIONS FOR USE
	DATE OF MANUFACTURE		KEEP AWAY FROM SUNLIGHT
	KEEP DRY	Σ	CONTAINS SUFFICIENT FOR N TESTS

[Basic Information]

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