Human Papillomavirus 5 High-risk Subtypes DNA Diagnostic Kit Manual
(PCR-fluorescence probing)

【Product name】

Human Papillomavirus 5 High-risk Subtypes DNA Diagnostic Kit

(PCR-fluorescence probing)

【Packaging specification】

20 tests/box

【Intended use】

The kit is used for detecting human papillomavirus 16, 18, 33, 52 and 58 subtypes infection in cervical exfoliated cells, and is not used for genotyping. The results are only for clinical reference and should not be taken as the only basis for diagnosis or exclusion of disease cases, and are not used for cervical cancer screening.

Human papillomavirus (HPV) can infect reproductive tract through many ways, which can lead to condyloma acuminatum, cervical lesions and even cervical cancer. It takes about 5-10 years from the persistent infection of high-risk HPV to the common cervical precancerous lesion and eventually develops into cervical cancer. Therefore, targeted detection of high-risk HPV has certain significance for the early diagnosis and treatment of cervical cancer. HPV16, 18, 52, 58 and 33 are the most common types of HPV high-risk infection and are closely related to the occurrence of cervical cancer. The primers designed of this kit can cover the detection of HPV16, 18, 33, 52 and 58, and the positive result only indicates the single or mixed infection of the five types and cannot distinguish them.

【Detection principle】

Based on HPV16, 18, 33, 52 and 58 gene sequences, this kit designs specific primers and fluorescent probes, and uses PCR- fluorescent probe method to detect the viral nucleic acids of HPV 5 subtypes in the sample, which can detect single type infection or mixed infection.

【Kit contents】

Contents		Specification	Quantity	Ingredients
Nucleic acid extraction reagent	HPV DNA extraction solution	1mL/vial	1	Chelex 100, Tris HCI, NaOH, Triton-100, NP-40, EDTA
PCR reagent	HPV 5 types PCR reaction solution	45μL/vial	20	Primers, probes, Dntp, DNA polymerase,UNG enzyme, buffer system
Quality controls	HPV 5 types positive control	30μL/vial	1	Inactivated HPV 16 subtype positive sample
	HPV 5 types negative control	30μL/vial	1	Inactivated HPV negative sample

Notes: different contents from different batches are not interchangeable.

Quality control instruction: HPV positive control and negative control are both collected from the clinical HPV16 positive and negative cervical specimens, which were treated by normal saline and centrifugation and then suspended. The quality controls were both inactivated already.

【Storage and validity】

Kit should be kept at -20±5℃ away from lights, avoid repeated freezing and thawing. It is valid for 6 months. Refer to the label for manufacture date and validity date.

Avoid leaving the reagent at room temperature for a long time after opening. Keep the remaining reagent in time after the test and store it at-20+5℃.

Transportation with ice packs in sealed foam boxes shall not exceed 7 days on the premise that the ice packs are not completely melted.

【Compatible instruments】

TIB-8600, ABI Prism 7500, Agilent Mx3000P

【Sample requirements】

1. Specimen collection: under aseptic conditions, insert the cervical brush into the cervical canal for 2cm, rotate for several circles and take out. Though a small amount of urine, blood and external drugs will not affect the performance of the kit, avoid these circumstances as much as possible when sampling.

2. Preservation: place the cervical brush specimen in the tube containing 1mL of normal saline, stir and squeeze it dry, and prepare the sample solution for examination. Specimens can be immediately used for testing or stored at -20℃ for 6 months.

3. Transportation: the specimen shall be transported at about 0℃.

【Procedure】

1. Sample treatment

(1). Vortex the sample solution and transfer 200μL into a 1.5mL centrifuge tube, centrifuge for 5 minutes at 10,000rpm, discard the supernatant and keep the precipitate.

(2). Thaw and vortex the positive and negative controls, pipet 10μL of each control and add omtp the 1.5mL centrifuge tube respectively.

(3). Add 50μL DNA extraction solution to sample precipitation and quality controls respectively, mix well and boil for 10 minutes, centrifuge for 5 minutes at 10,000rpm, and keep the supernatant for testing.

2. PCR amplification

(1). Pipet HPV5 types PCR reaction solution 5μL into sample extraction solution and supernatant of quality control respectively.

(2). Centrifuge the PCR reaction solution at 6,000rpm and put into the fluorescent PCR instrument to amplify as per below sequence:

Cycle Parameters:

Stage 1	50° C	---- 2 min
Stage 2	94° C	---- 3 min
Stage 3		
10 cycles of		
	94° C	---- 15 sec
	53° C	---- 1 min
Stage 4		
30 cycles of		
	94° C	---- 15 sec
	53° C	---- 45 sec

Fluorescein setting: HPV5-FAM, TAMRA
Fluorescein signal collection: stage 4 53° C --- 45 sec
Reaction volume: 50μL

(3). Results analysis: the results will be automatically saved after the reaction is completed. The Start, End and Threshold values of Baseline can be adjusted according to the analyzed image (the user can adjust according to the actual situation, the Start value can be set at 1-2. The End value can be set at 5-10, and the amplification curve of negative control can be adjusted to be straight or lower than the threshold line). Click "Analysis" to automatically obtain the analysis results and view the results in the "Report" interface. The actual Ct value is Ct value of test report plus 10.

3. Quality control

(1) Negative control: no typical s-type amplification curve or Ct value.
(2) Positive control: typical s-type amplification curve and Ct value is equal or less than 35.1.
(3) The above requirements must be met simultaneously in the same experiment, otherwise, the experiment is invalid and retest is needed.

4. Results judgments

(1) If the sample has no typical s-type amplification curve or Ct value is higher than 35.1, then the sample is judged to be HPV 5 subtypes negative.
(2) If the sample shows a typical s-type amplification curve, and the Ct value is ≤35.1, then the sample is judged to be HPV 5 subtypes positive.

【Positive judgment value】

Through the analysis of clinical test results and ROC curve method, the Ct reference value of this kit is judged as 35.1.

【Results interpretation】

If the sample shows a typical s-type amplification curve, and the Ct value is ≤35.1, it is possible that the sample is infected by single or mixed subtypes of HPV 16, 18,33,52 and 58

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【Detection Limitation】

Specimen concentration that is lower than the LOD of this kit cannot be detected.

【Kit Performance】

1. LOD (limit of detection) of this kit for HPV 16, 18, 33, 52 and 58 subtypes are 1.0×10^3copies/mL.
2. Accuracy: no difference between CV within the same batch and CV among different batches.
3. Specificity analysis: common pathogens in genitourinary system such as Mycoplasma trachomatis (CT), Ureaplasma urealyticum (UU), Neisseria gonorrhoeae (NG) and other common types of HPV are all negative detected by this kit. Blood collected from samples and common external drugs will not interfere with the results.
4. Clinical comparative experiment: through the verification of 596 clinical samples, the kit has very strong consistency with similar products on the market, and the coincidence rate of negative and positive of each subtype is above 98%.

【Warnings and Precaution】

This product is for in vitro diagnosis purpose only.

1. To avoid any potential biological hazards in the sample, samples should be regarded as contagious and avoid contact with skin and mucous membrane. Sample handling is recommended to be in the biological safety cabinet which can prevent aerosol outflow, and the used test tubes and pipet-tips for the operation should be sterilized before being discarded. The handling and disposal of specimens should meet the requirements of relevant laws and regulations: “The General Guidelines for Biosafety of Microbial Biomedical Laboratories” and “The Regulations on the Management of Medical Wastes” issued by the Ministry of Health.
2. Although the negative and positive quality control samples have been proved that the virus has been inactivated, no known test method can completely ensure that human-derived substances do not contain infectious substances after inactivation. All human-derived substances may be potentially contagious and should be treated as contagious during operation.
3. Lab personnel must be professionally trained. PCR experiments should be carried out in the sample processing room, PCR sampling room, PCR amplification room and each room should be relatively separated. Human and experimental materials should be moved unidirectionally from sample processing room to PCR sampling room to PCR amplification.
4. Provide negative pressure ultra-clean biosafety cabinet for reagent and sample preparation.. During experiments, please wear lab coats and disposable gloves and use self-unloading pipettes. Lab coats from PCR processing room and hybridization room should be separated.
5. Carry out quality control for each experiment.
6. Specimen treatment: when pipetting 50μL DNA extraction solution into the sediments, mix well before pipetting since there are some water insoluble granular materials in the DNA extraction solution. Use the sterilized scissor to cut a small part off from the pipet filter if there is any jam in the filter causing pipetting problems.
7. The PCR reaction tubes, centrifuge tubes and pipet-tips used for PCR reaction should be autoclaved and used as disposable.
8. The pipet-tips used in the experiment should be dismounted directly into the waste tank containing sanitizer and discarded after sterilized together with other waste materials.
9. After the experiment, sterile the workbench and pipette with 10% chloric acid or 70% alcohol or ultraviolet light.

【References】

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2. Bao Yanping, Li Ni, Wang He, Qiao Youlin, Meta-analysis of cervical HPV type distribution in Chinese women, Chinese Journal of Epidemiology 2007,28(10):941-942.
3. Geng Jianxiang, Wang Xubo, Human Papillomavirus Detection and Its Clinical Application. People's Health Publishing House. 2009

【Manufacturer】

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