TRIPLEX INTERNATIONAL BIOSCIENCES(CHINA) CO., LTD.

Human Papillomavirus 13 High-risk Subtypes DNA Diagnostic Kit Manual

(PCR-fluorescence probing)

【Product Name】

Human Papillomavirus 13 High-risk Subtypes DNA Diagnostic Kit
(PCR-fluorescence probing)

【Packaging Specification】

20 tests/box

【Intended Use】

The kit is used for qualitative detecting human papillomavirus 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 subtypes infection in urethral secretion, cervical exfoliated cells, and verrucous cells samples. It is not used for genotyping and can be used for the auxiliary diagnosis of HPV infection and the prognosis and treatment of related diseases. The results are only for clinical reference and should not be taken as the only basis for diagnosing or excluding of disease cases. The kit is not used for cervical cancer screening.

The existence and long-term infection of human papillomavirus (HPV) is closely related to various benign and malignant hyperplasias of skin and mucosa, specifically the occurrence and development of cervical cancer. The gene sequences of various viruses are highly correlated with their biological behaviors. Different genotypes of HPV have different pathogenic risks. The detection and typing of HPV DNA are of great value for understanding the related diseases, judging the prognosis, and guiding the treatment, especially for the cancer prediction of female genital tract tumors.

There are more than 110 subtypes of HPV identified in the world today. More than 20 subtypes of HPV have been isolated from cervical cancer tissues. According to their pathogenicity, they are divided into two types: high-risk type and low-risk type. The International Association for Cancer Research classified HPV6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP 6108 as low-risk types. They mainly cause genital, perianal skin, lower vagina exophytic condyloma lesions, flat condyloma lesions, and low-grade cervical intraepithelial neoplasia (CIN I) that are transient and can be reversed naturally. The 15 high-risk subtypes, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82, mainly lead to CIN Ⅱ-Ⅲ lesions and cervical cancer. CIN I with persistent high-risk HPV infection can easily develop to CIN Ⅱ-Ⅲ. According to the current clinical and epidemiological investigation data in China, more than 20 subtypes have become the major epidemic strains, including the five low-risk types, HPV6, 11, 42, 43, and 44, and the high-risk types such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82, and so forth.

The kit designs specific primers and probes for HPV genome L1 region, and adopts real-time fluorescence PCR technology to detect 13 common high-risk HPV genotypes, including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The designed primers can cover the above 13 subtypes' detection, and the positive results only indicate single-type or mixed -type infections in the 13 subtypes, which cannot be distinguished,

【Detection Principle】

The product adopts real-time fluorescent PCR technology, designs specific primers and fluorescent-labeled probes for L1 regions of 13 subtypes of HPV high-risk genes, and amplifies explicitly and detects 13 subtypes of HPV high -risk gene fragments. Real-time fluorescence PCR is to realize the initial template's quantitative and qualitative analysis by real-time detection of each circulating product's florescence signal in the PCR amplification reaction. During the real-time fluorescence quantitative PCR reaction, a fluorescent chemical was introduced. In the progress of the PCR reaction, the PCR products accumulate continuously, and the fluorescence signal intensity increases proportionally. After each cycle, a fluorescence intensity signal is collected to monitor the change of product quantity through the change of fluorescence intensity, thus obtaining a fluorescence amplification curve.

【Kit Contents】

Contents		Specification	Quantity	Ingredients
Nucleic acid extraction reagent	HPV DNA extraction solution	1mL/vial	1	Chelex 100, Tris HCl, NaOH, Triton-100, NP-40, EDTA
PCR reagent	HPV 13 types PCR reaction solution	45μL/vial	20	Primers, probes, dNTP, DNA polymerase, UNG enzyme, buffer system
Quality controls	HPV 13 types positive control	30μL/vial	1	Inactivated HPV 16 subtype positive sample
	HPV 13 types negative control	30μL/vial	1	Inactivated HPV 13 subtype negative sample

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

Notes: different contents from different batches are not interchangeable.

【Storage and validity】

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

Notes:

1. Kits are still valid for use after opening in 8 hours. Avoid long time opening in case of evaporation.
2. Kits are still valid for use when sealing in foam boxes with ice packs for 7 days, which meet the long term transportation need.
3. Kits are still valid for use after six times repeated freezing and thawing. Please avoid unnecessary freezing and thawing in clinical use.

【Compatible instruments】

Agilent Stratagene Mx3000P, ABI Prism 7500, TIB-8600.

【Sample requirements】

1. Collection

1) For the urethral secreta, insert the cotton swab into the urethra under sterilized environment and gently rotate for a few circles, stay for a few seconds, and take it out.

2) For cervical exfoliated cell, insert the cervical brush into the cervix 2cm under sterilized environment and gently rotate for a few circles. , stay for a few seconds, and take it out..

3) For verrucous cell, use a sterile cotton swab to scrape the epithelium from the verruca.

2. Storage

Keep the cervical brush specimen or cotton swab specimen in the sterilized tube with 1mL normal saline. Stir and squeeze the brush or swab, keep the remaining solution for immediate detection, or preserve the solution at -20°C for later detection. Valid for 6 months.

3. Transportation

Specimens should be transported and carried out at around 0°C.

(Interference test proves that, a small amount of urine, blood, and drugs for external use have no effects on the kit's performance. Nevertheless, avoid these interfered factors as much as possible during clinical specimen collection).

【Procedure】

1. Specimen Treatment

1) Spin down the specimen solution tube and pipette 200μL into 1.5mL centrifuge tube. Centrifuge for 5 min at 10,000rpm

and discard the supernatant. (Rinse the specimen one or two times with normal saline if there are any impurities)
2) Thaw the positive control and negative control and then spin down briefly, pipette 10μL of each control into two 1.5 mL centrifuge tubes respectively.
3) Pipette 50μL DNA extraction buffer into the specimen tube, positive control tube, and negative control tube respectively. Boil for 10 min and centrifuge for 5 min at 10,000rpm. Keep the supernatant for detection.

2. PCR amplification

1) Pipette 5μL of the sample extraction solution or quality control extraction into the HPV 13 types PCR reaction solution respectively.

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

Cycle Parameters:

Stage 1	50°C ---- 2 min
Stage 2	93°C ---- 3 min
Stage 3	10 cycles of
	93°C ---- 45 sec
	51°C ---- 60 sec
Stage 4	30 cycles of
	93°C ---- 30 sec
	51°C ---- 45 sec

Fluorescein setting: HPV 13 - FAM, TAMRA
Fluorescence signal collection: stage 4 : 51°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) HPV 13 negative control: no typical s-type amplification curve or Ct value.
2) HPV 13 positive control: typical s-type amplification curve and Ct value ≤ 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 13 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 13 subtypes single or mixed positive.

【Positive judgment value】

Through the analysis of clinical test results, the Ct reference value of this kit is judged as 35.1.t.

【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 HPV 13 subtypes.

【Detection limitation】

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

【Kit performance】

1. Sensitivity: the sensitivity of this kit is 1×103copies/mL for the HPV 13 subtypes.
2. Specificity: the kit has no cross reaction with the urogenital tract pathogens including treponema pallidum, mycoplasma hominis, neisseria gonorrhoeae, chlamydia trachomatis, ureaplasma urealyticum and other common HPV subtypes such as HPV 6, 11, 42, 43, 44 positive samples.
3. Accuracy: The Ct value’s variation coefficient (CV value) of the kit’s detection result on HPV13 types of samples is less than 10%.

【Warnings and precaution】

1. This product is for in vitro diagnosis purpose only.
2. In order to avoid any potential biological hazards in the sample, samples used for the test should be regarded as contagious and avoid contact with skin and mucous membrane. Sample handling is recommended to be in the biological safety cabinet that can prevent aerosol outflow. The used test tubes and pipette-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.
3. 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 any infectious substances after inactivation. All human-derived substances may be potentially contagious and should be treated as contagious during operation.
4. 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 follow the unidirectional workflow from the sample processing room to the PCR sampling room and, eventually, the PCR amplification room.
5. 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 the PCR processing room and the hybridization room should be separated.
6. Carry out quality control for each experiment.
7. Specimen treatment: Mix the DNA extraction solution well before adding 50μL DNA extraction solution into the sediments 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 tip if there is any jam in the filter causing pipetting problems.
8. The PCR reaction tubes, centrifuge tubes, and pipette-tips used for PCR reaction should be autoclaved and used as disposable.
9. The pipette-tips used in the experiment should be dismounted directly into the waste tank containing sanitizer and discarded after sterilization together with other waste materials.
10. After the experiment, sterile the workbench and pipette with 10% chloric acid or 70% alcohol, or ultraviolet light.

【Manufacturer】

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