**18 human papillomavirus (HPV) nucleic acid test kit (PCR-fluorescent probe method) instructions**

**【Product Name】**

*Human papillomavirus*（18 types HPV）DNA qPCR detection Kit

**【Package specification】**

32 servings/box

**【Intended use】**

This kit is suitable for the qualitative detection of human papillomavirus (including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 26, 53, 66, 73, 82) DNA in female cervical exfoliated epithelial cells, and the specific clinical application, clinicians need to combine with the actual situation of the case The results of this kit should not be used as the only basis for clinical diagnosis.

This kit is not intended for 1) screening patients with ASC-US (atypical squamous epithelial cells of undetermined significance) findings on cervical cytology to determine the need for colposcopy (referred to as ASC-US population triage use); 2) for women aged 30 years and older, screening for cervical cancer by testing for high-risk HPV infection in combination with cervical cytology, this This test is used to guide the management of patients by combining the assessment of cytologic history and other risk factors, as well as the requirements of clinical diagnosis and screening guidelines (referred to as combined cervical cancer screening use); 3) for women of a certain age, cervical cancer screening is performed by testing for the presence of high-risk HPV infection, and this test is used to guide the management of patients by combining the assessment of cytologic history and other risk factors, as well as the requirements of clinical diagnosis and screening guidelines (referred to as cervical cancer combined screening use). It is used to guide patient management (referred to as primary cervical cancer screening use).

HPV infecting the genital and anal tracts can be classified into two categories, low-risk and high-risk, depending on the pathogenicity or cancer risk of each genotype. HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 are high-risk genotypes, while HPV 26, 53, 66, 73 and 82 are intermediate risk genotypes. This kit typed HPV16 and 18, and grouped 11 high-risk genotypes such as HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 5 intermediate risk genotypes such as HPV26, 53, 66, 73, 82, respectively.

**【Test Principle】**

The kit is based on the principle of fluorescent PCR method, combined with Taqman fluorescent probe technology to design specific primers and probes for conserved gene fragments of human papillomavirus and conserved regions of the human genome, respectively, and detect HPV DNA and human genomic DNA simultaneously in a fully closed amplification system using multiplex fluorescent PCR technology.

**【Main reagent composition】**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Serial number | Grouping | Main components | Quantity | Specification |
| 1 | HPV PCR reaction solution | Primers, Probes, PCR Buffer, dNTP, MgCl2, DNA polymerase, UDG enzyme, Taq-Antibody | 1 | 1.44 mL/tube |
| 2 | HPV-positive control | 2000 copies/μL of a mixture of 18 HPV plasmids and human genomic plasmids | 1 | 0.20 mL/tube |
| 3 | HPV negative control | 2000 copies/μL of human genomic plasmid | 1 | 0.20 mL/tube |
| 4 | Instruction manual | // | 1 | 1 |

Note: Different batch components cannot be mixed.

Prepare your own saline (0.9% NaCl).

Sampling and extraction components are not included in this kit.

**【Storage conditions and expiration date】**

-Store below 18℃ away from light.

The recommended number of repeated freeze-thawing is less than 6 times.

The expiration date is 12 months, and it is recommended to use up the product within 4 weeks after opening the cap.

**【适用仪器】**

ABI 7500荧光PCR仪。

**【Specimen requirements】**

1. female cervical exfoliated cells, cervical swabs.

2. Swab sample collection (non-menstrual period is required, pelvic examination, vaginal ultrasound, vaginal irrigation and drug application are prohibited within 24 hours prior to sampling).

Cervical swab: fully expose the cervix, using the ectocervix as the center of the circle, and gently rotate a sterile cotton swab 2 times to wipe away the secretions at the epithelial junction of the squamous column epithelium of the ectocervix button and inside the ectocervix.

Cervical exfoliated cells: fully expose the cervix, take the ectocervix as a circle, and rotate 3-4 turns with gentle pressure using a cervical brush at the epithelial junction of the squamous column epithelium of the ectocervix and within the ectocervix, stay for 10 seconds and then remove.

3. The swab or cervical brush after sampling is placed into a sample collection tube containing 1 mL of saline, and the liquid is the sample to be tested after full shaking and washing of the swab or cervical brush.

4. Storage and transport of samples.

Samples can be used immediately for testing or stored at 2~8°C (no more than 7 days), samples can be stored for up to 12 months below -18°C, and for long term storage below -70°C. Samples are transported over long distances using ice jars with ice or foam boxes with ice packs and then sealed. Frozen samples need to be thawed at room temperature and then processed to avoid repeated freezing and thawing. In-hospital transport is done at room temperature.

**【Inspection method】**

**1. Reagent preparation (reagent preparation area)**

**1.1 Remove the HPV PCR reaction solution from the kit, thaw it at room temperature, thaw it sufficiently, mix it with gentle shaking, and set aside for brief centrifugation.**

**1.2 According to the number of samples to be examined (n), each reaction tube was divided into 45 μL of HPV PCR reaction solution, and a total of (n+2) tubes were divided, where '2' in (n+2) refers to HPV-positive control and HPV-negative control.2. Sample processing (sample processing area)**

**It is recommended to use verified nucleic acid extraction reagents (filing number: SuTong械备XXXXXXXXXX) and perform nucleic acid extraction according to the instructions as follows.**

**2.1 Transfer 200 μL of liquid into a 1.5 mL tube containing 1000 μL of saline, centrifuge at 12000 rpm for 2 minutes, and aspirate the supernatant using a pipette.**

**2.2 Add another 1 mL of saline to the 1.5 mL tube, shake until no visible precipitate is visible, centrifuge at 12,000 rpm for 2 minutes, and aspirate the supernatant using a pipette.**

**2.3 Take two other 1.5mL tubes and add 50μL of HPV negative control and HPV positive control respectively.**

**2.4 Add 50 μL of inverted and mixed DNA extract to the sample tube in 2.2, the HPV-negative control and the HPV-positive control tube in 2.3, respectively.**

**Note: DNA extracts should be inverted and mixed in time before adding and during use.**

**2.5 Shake on a vortex shaker to break up the precipitate, dry bath at 90±5℃ or water bath for 10±2 minutes, centrifuge at 12000rpm for 2 minutes, and use the supernatant for PCR reaction.**

**Note: The extracted DNA should be used for the assay in time, otherwise it should be stored below -18°C (no more than 18 months, freeze-thaw no more than 3 times), and when used again, the extracted DNA should be fully melted, mixed and centrifuged instantaneously, and the supernatant should be taken for PCR reaction.**

**3. Addition of samples (sample processing area)**

**Add 5 μL of the treated HPV negative control, the sample to be tested and the HPV positive control to the PCR reaction tube with prepared reagents, tighten the cap and centrifuge the tube transiently at low speed.**

**Note: Do not touch the sediment at the bottom of the tube during sample aspiration.**

**4. PCR amplification (PCR amplification zone)**

**4.1 Sample setup: Set the sample number, HPV negative control and HPV positive control according to the sample type.**

**4.2 Fluorescence channel selection: select FAM, JOE, TAMRA, TEXAS RED , CY5 channels for each sample, Quencher fluorescence (Quencher) is set to None, and reference fluorescence (Passive Reference) is set to None.**

**4.3 Reaction conditions setting**

|  |  |  |  |
| --- | --- | --- | --- |
| Steps | Temperature (℃) | Time (seconds) | Number of cycles (times) |
| 1 | UDG enzyme reaction | 37 | 300 | 1 |
| 2 | Pre-mutability | 95 | 300 | 1 |
| 3 | Sex Change | 95 | 10 | 45 |
| Annealing, extension and detection of fluorescence | 60 | 60 |
| HPV16 detection channel: FAM;HPV18 detection channel: JOE;HPV31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 detection channel: TEXAS RED. HPV26, 53, 66, 73, 82 detection channel: CY5;Internal reference (IC) detection channel: TAMRA. |

The reaction volume is set to 50 μL.

4.4 Save the file and run the program.

5. Result analysis

After the reaction, set Baseline to 3-15 (Baseline Cycle can be varied within a certain range according to the actual situation), the fluorescence threshold (Threshold) is set based on the principle that the threshold line just exceeds the highest point of the negative control amplification curve (irregular noise line) and the Ct value is shown as Undetermined; the general FAM, TEXAS RED channel fluorescence threshold 2.0E+04 to 2.0E+05, JOE, CY5 channel fluorescence threshold 1.0E+04 to 2.0E+05, TAMRA (IC) channel fluorescence threshold 1.0E+04 to 1.0E+05. recommended to set the FAM, TEXAS RED channel fluorescence threshold of 5.0E+04, JOE, CY5 channel The fluorescence threshold of FAM and TEXAS RED channel is 5.0E+04, JOE and CY5 channel is 4.0E+04, and TAMRA (IC) channel is 2.0E+04. The results are analyzed automatically using the instrument supporting software.

6. Quality control

6.1 HPV negative control: FAM, JOE, TEXAS RED and CY5 channels all had no obvious S-shaped amplification curve, and the Ct values all showed Undetermined; TAMRA channels all had typical S-shaped amplification curves, and the Ct values were all ≤ 34.00.

6.2 HPV positive control: FAM, JOE, TEXAS RED and CY5 channels all have typical S-type amplification curves with Ct values ≤ 34.00; TAMRA channels all have typical S-type amplification curves with Ct values ≤ 34.00.

6.3 The above two conditions must be satisfied at the same time, otherwise this test is invalid.

【Reference value range】.

1. FAM, JOE, TEXAS RED and CY5 channels all have no obvious S-type amplification curve, and the Ct values all show Undetermined or 38.00 < Ct value, which is negative for HPV.

2. Any of the FAM, JOE, TEXAS RED and CY5 channels had obvious S-type amplification curves and Ct values ≤ 38.00 were positive for the HPV type or group of the corresponding channel.

[Interpretation of test results].

Under the premise of validity of the experiment, the test results of HPV of the samples were judged as follows.

|  |  |  |
| --- | --- | --- |
| Passage | Ct≤38.00 | 38.00＜Ct值或Undetermined |
| CY5 | HPV16 positive | HPV16 negative |
| JOE | HPV18 positive | HPV18 negative |
| FAM | HPV31、33、35、39、45、51、52、56、58、59、68At least one positive | HPV31、33、35、39、45、51、52、56、58、59、68negative |
| TEXAS RED | HPV26、53、66、73、82At least one positive | HPV26、53、66、73、82negative |
| 1. 1. For samples positive for any one HPV type or group, this test sample is reported as positive for the corresponding type or group of HPV, and the rest of the HPV types or groups are negative.
2. 2. For samples that are negative for all HPV types or groups, the samples are further analyzed according to the Ct value of the IC channel.
3. 1) IC Ct value ≤ 37.00 was reported as negative for all 18 HPV types.
4. 2) IC Ct values are Undetermined, then the PCR reaction is a failure.
5. (3) IC 37.00 < Ct value ≤ 45.00, and suggest that sufficient samples may not have been collected, it is recommended to resample.
6. 3, PCR reaction failure is recommended to re-measure, if there is a fluorescent signal, the results are determined as above, otherwise resample.
7. HPV negative results then this test sample for the kit to detect HPV type DNA negative or below the detection limit of the kit.
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**Limitations of the test]**

**1. This kit is suitable for clinical auxiliary diagnosis, and the clinical diagnosis and treatment of patients should be considered in conjunction with other medical tests.**

**2. False-negative or false-positive results may occur when samples are not collected, handled, transported, or stored properly.**

**3. Analysis of the possibility of false negative results**

**Improper sample collection, transfer, handling, or low sample concentration may lead to false negative results.**

**Variations in the target sequence to be tested or other causes of sequence alteration may lead to false negative results.**

**Split sample collection from the same patient and multiple sites will reduce the likelihood of false negative results.**

**Product performance indicators]**

**1. The minimum detection limit for all 24 types of HPV in this kit can reach 500 copies/reaction.**

**2. 100% compliance rate when using corporate negative and positive references for the kit.**

**3. The coefficient of variation of the intra-batch precision of the kit is less than 5%.**

**4. The specificity test shows no cross-reactivity with other pathogens (including CT, MG, MH, UU, UP, NG, HSV2, TV, CA) in the genital tract and urinary tract secretions.**

**【Caution】.**

**1. This kit is for in vitro testing only.**

**2. Please read this instruction manual carefully before using this kit. The experimental operator should have received professional training in gene amplification or molecular biology method detection and have relevant experimental operation qualification, and the laboratory should have reasonable biosafety preparedness facilities and protection procedures.**

**3. The entire experimental process should be carried out in three areas (reagent preparation area, sample processing area, PCR amplification area), please strictly partition the operation; instruments, equipment, consumables and work clothes in each area are dedicated, no cross-use, please clean the bench immediately after the experiment.**

**4. The product should be fully melted at room temperature, mixed and instantaneously centrifuged at low speed before use.**

**5. Negative and positive controls should be set for each experiment. Do not mix reagents of different batches and use the kit within the expiration date.**

**6. The samples should be as fresh as possible, and the extraction process should prevent DNA contamination and DNA degradation caused by improper operation.**

**7. DNA samples stored below -18°C should be fully melted, mixed and centrifuged at room temperature before addition.**

**8. Avoid air bubbles as much as possible when dispensing the reaction solution, and check whether each PCR reaction tube is tightly capped before putting it into the amplification instrument to avoid contamination of the instrument and the environment.**

**9. The test samples involved in this kit should be regarded as infectious substances, and the operation and handling should comply with the relevant requirements of the General Guidelines for Biosafety in Microbiological and Biomedical Laboratories of the Ministry of Health and the Regulations on the Management of Medical Waste.**

**【Reference】.**

**1. Li JM. Real-time fluorescence PCR techniques [M]. Beijing; People's Military Medical Press, 2007.**

**2. Roche Sponsor Presentation,cobas ®HPV Test Microbiology Devices Panel P100020/S008 March 12, 2014.**

**3. IRAC Human papillomavirus 2014,mono100B-11.**

**4. WHO Human papillomavirus vaccines:WHO position paper,May 2017.**

**5. State Drug Administration, Guidelines for technical review of human papillomavirus (HPV) nucleic acid testing and genotyping reagents, 2015.**

**【Manufacturer】.**

**Registrant/manufacturer name: Jiangsu Ruibo Biotechnology Co.**

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**Production License No.: Su Food and Drug Administration Machinery Production Xu 2019XXXX No.**

**Medical device registration certificate number/product technical requirement number**

**State Armament Note Approval 2019340XXXX**

**[Instruction approval date and modification date]**

**The approval date of the manual is XX/XX/201X.**