Human Papillomavirus Subtypes 16 and 18 DNA Diagnostic Kit Manual
(PCR-fluorescence probing)

【Product name】

Human Papillomavirus Subtypes 16 and 18 DNA Diagnostic Kit

(PCR-fluorescence probing)

【Packaging specification】

48 tests/box

【Intended use】

The kit is suitable for detecting HPV subtypes 16 and 18 infection in clinical samples and the positive result indicates that the clinical samples carry HPV subtype 16 and 18 single or mixed infection which cannot be distinguished from each other. The results are only for clinical reference and should not be taken as the only basis for diagnosis or exclusion of disease cases

【Detection principle】

This kit selects HPV16, 18 gene sequences, designs specific primers and fluorescent probes, and adopts PCR- fluorescent probe method to detect HPV16, 18 single infections or mixed infection in samples.

【Kit contents】

Contents	Quantity	Specification	Ingredients
HPV 16,18 PCR Reaction Reagent	1 vial	2108μL/vial	Primers, probes, dN(U)TPs, buffer system
Taq/UNG Enzyme	1 vial	53μL/vial	Taq DNA polymerase, UNG enzyme
Mineral Oil	1 vial	1200μL/vial	Mineral oil
HPV 16,18 positive control	1 vial	400μL/vial	Inactivated HPV16 subtype positive specimen
HPV 16,18 negative control	1 vial	400μL/vial	Inactivated HPV negative specimen
Internal control	1 vial	240μL/vial	Plasmids containing target gene fragment

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 that were inactivated already.

【Storage and validity】

Kit should be kept at -20±5℃ away from lights, avoid repeated freezing and thawing. It is valid for 8 months.

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, HHT GA2100

【Sample requirements】

The sample of this kit is from HPV DNA, which is prepared by nucleic acid extraction reagents (QIAmp DNA Blood Mini Kit manufactured by Qiagen, Item No.51104). 5uL internal control is added into the sample for extraction.

The prepared HPV DNA shall be stored at 2-8℃ for no more than one day, at -20±5℃ for no more than one month, and shall not be frozen and thawed repeatedly.

【Procedure】

1. **Reagent preparation**

   Prepare the reaction solution according to sample number n (sample number n = number of samples to be tested + 2 quality controls). Pipet n x 43.9μL HPV16, 18 PCR reaction solution and n x 1.1μL Taq/UNG enzyme respectively and mix them evenly. Aliquot them into PCR reaction tubes by 45μL/tube. Add 25μL mineral oil as PCR reaction solution tube.

2. **Treatment of samples and quality controls and sample adding**

   DNA of samples and quality controls are prepared by nucleic acid extraction reagents. Before extraction, add 5μL of internal control to the sample and quality control for extraction.

   Sample addition: add 5μL of HPV sample and quality control into PCR reaction tube. Spin them down briefly then put into the PCR instrument and amplify as per below sequence.

   Cycle Parameters:

   Stage 1 37° C ---- 2 min
   Stage 2 94° C ---- 2 min

   Stage 3
   40 cycles of
   94° C ---- 15 sec
   55° C ---- 45 sec

   HPV detection fluorescein: FAM
   Internal control detection fluorescein: HEX (use JOE channel if HEX is not available)
   Fluorescein signal collection: stage 3 55° C --- 45 sec
   Reaction volume: 50μL

   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 3-5. The End

value can be set at 12-15, 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.

3. Quality control

(1) Negative control: HPV (FAM) has no typical s-type amplification curve or Ct value, and Ct value of internal control (HEX) is less than 40 with nice amplification curve.
(2) Positive control: HPV (FAM) has typical s-type amplification curve and Ct value is equal or less than 35.1. Ct value of internal control (HEX) is less than 40 with nice amplification curve.
(3) The above requirements must be met simultaneously in the same experiment, otherwise, the experiment is invalid and retest is needed.

4. Results judgements

The Ct value of each sample is obtained by automatic analysis by the instrument.
(1) if the sample HPV (FAM) has no typical s-type amplification curve or Ct value is higher than 35.1, the Ct value of internal control (HEX) is less than 40 with a nice amplification curve, then the sample is judged to be HPV subtype 16 and 18 negative.
(2) if the sample HPV (FAM) shows a typical s-type amplification curve, and the Ct value is $\leqslant$ 35.1, and the Ct value of internal control (HEX) is less than 40, then the sample is judged to be HPV subtype 16 and 18 positive.
(3) If the sample HPV (FAM) has no typical S-type amplification curve or Ct value $>$ 35.1, and the internal control (HEX) has no amplification curve or Ct value, the experiment fails. And it is suggested to recheck the extraction and amplification process.
Notes: HPV(FAM)35.1＜Ct＜40 is detection grey area and retest is required. If the result is still 35.1＜Ct＜40, judge the result according to Ct≤35.1

【Reference 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】

When the result is HPV16 and 18 positive, it is possible that the sample is infected by HPV16 or HPV 18 subtype, or infected by both subtypes simultaneously.

【Detection Limitation】

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

【Kit Performance】

1. LOD (limit of detection) of this kit for HPV 16 and 18 subtypes are both 1.0×10^3copies/mL.
2. Accuracy: CV within the same batch and CV among different batches are both $\leqslant$ 5%
3. Specificity analysis: blood collected from samples and common external drugs will not interfere with the results. 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.

【Warnings and Precaution】

1. This product is for in vitro diagnosis purpose only.
2. Please read the full text of the instruction carefully before the experiment. The experiment should be conducted by experienced or trained laboratory personnel.
3. Use latex gloves or thin film gloves when handling the PCR tubes.
4. There are fluorescence probes in the PCR reaction solution, avoid unnecessary repeated freezing and thawing and keep the PCR solution away from lights.
5. Thoroughly thaw the reagents and pin them down briefly before using.
6. Sterilize centrifuge tubes and pipet tips in high temperature and high pressure before being used.
7. Dispose the pipet tips into the 10% sodium hypochlorite wasted solution vat and sterilize with other wastes.
8. Sterilize the biohazard safety cabinet by UV lights. After the experiment, clean the biohazard cabinet and pipets with 10% pasteurization, then use 75% ethyl alcohol for cleaning after 10 min.
9. Do not mix-use the reagents from different batches. Use this kit before its expiration.

【References】

1. Qin Yiqiu, Research Progress on Correlation between Human Papillomavirus and Human Tumor, Laboratory Medicine. 2009,24(4): 311-315.
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

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