# HPV DNA PCR Detection Kit（Lyophilized）

## Use Manual

Cat. No.: RH016G

| Label | Description | Label | Description |
|---|---|---|---|
|  | Contains sufficient for <N> tests |  | In vitro diagnostic |
|  | Batch number |  | Consult instruction before use |
|  | Date of manufacturing |  | European Anthorized Representative |
|  | Date by which the device should be used |  | Temperature limitation |
|  | Name and address of Manufacturer | | |

**【PRODUCT NAME】**

HPV DNA PCR Detection Kit（Lyophilized）

**【SIZE】**

48test/kit; 50test/kit

**【INTENDED USE】**

This product is intended for the qualitative detection of 15 kinds of high-risk human papillomavirus (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) DNA in exfoliated cells from females' cervix, and also for the subtype identification of HPV16 and HPV18.

Human Papillomavirus (HPV) belongs to the papillomavirus family. It is a small molecule, non-enveloped, circular double-stranded DNA virus with a genome length of about 8000 base pairs (bp), divided into three functional regions, namely the early transcribed region (E region), the late transcribed region (L region) and the non-transcribed region (long control region, LCR). The virus is not only host-specific, but also tissue-specific. It can only infect human skin and mucosal epithelial cells, causing various papillomas or warts of human skin and proliferative damage to the epithelium of the reproductive tract. HPV that infects the reproductive tract and anus can be divided into low-risk type and high-risk type according to the specific pathogenicity or carcinogenic risk of each genotype. Genital tract HPV infection is common among women with a history of sexual life. According to statistics, 70% to 80% of women will have HPV infection at least once in their lifetime, but most infections are self-limiting. High-risk HPV DNA can be detected in 99.7% patients with cervical carcinoma. HPV16 and 18 are the two most dangerous high-risk subtypes of human papillomavirus.

Base on the study results by WHO International Agency for Research on Cancer (IARC) and other international organizations, the 13 kinds of genotypes including HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 are classified as high-risk HPV, and the 5 kinds of genotypes including HPV26, 53, 66, 73, 82 are classified as medium-risk HPV. This diagnostic kit chooses the above 13 kinds of high-risk genotypes and 5 kinds of medium-risk genotypes to be the target genotypes, in order to guarantee that the kit is capable of cervical carcinoma screening and risk evaluation. Moreover, it is clearly indicated that the females at the age of 30 or above who have no abnormal cervical cytology but the HPV detection is

positive, especially for HPV16 and HPV18 infected females, should get vaginoscopy immediately. Therefore, this diagnostic kit can be used for detection of the 18 kinds of target genotypes and also subtype identification of HPV16 and HPV18.

## 【PRINCIPLE OF DETECTION】

This kit is based on the conserved region of HPV E gene as the target gene, using multiple PCR amplification, and multi-probe technology for four-channel (FAM, HEX, ROX, CY5) fluorescence detection analysis method,detecting 18 genotypes of high-risk HPV and subtype identification of HPV16 and HPV18. During template amplification, Taqman probes will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase. The separation of the fluorescent reporter gene and the quencher gene enables the fluorescent signal to be detected by the instrument arrive. CY5 channel detects HPV-16 type, FAM channel detects HPV-18 type, ROX channel detects other 16 types high-risk HPV without subtype identification and HEX channel detects human reference genes. The internal control is used in the kit for quality control from the beginning of sample collection to avoid false negative results.

## 【PRODUCT CONTENTS】

| Component | Volume | Package | Ingredients |
|---|---|---|---|
| HPV PCR Mix (Lyophilized) | 20μl×48 | 6×0.2ml or 0.1ml 8 well-strip tube | Contains primers and fluorescent probes, dNTPs, $MgCl_2^+$,Taq DNA polymerase |
| | 1000uL | 1×bottle | |
| Dissolving solution | 1200μl | 1×1.5ml tube | / |
| Negative Control | 200μl | 1×1.5ml tube | 0.9%NaCl |
| Positive Control (Lyophilized) | 40μl×2 | 2×0.2ml tube (Lyophilized) | Plasmid containing gene of HPV16,18,52 types and IC gene specific fragments |

Note:

1. The components in the kits of different batch numbers are not interchangeable.
2. Prepare your own experimental consumables: tubes, micropipettes, etc. of applicable specifications.

## 【STORAGE & SHELF LIFE】

Stored：2~30℃. And the kit is stable for 12 months

## 【INSTRUMENTS】

Real-time fluorescent PCR instrument with four fluorescent channels(FAM, HEX, ROX, CY5), such as ABI7500, Roche LC480/COBAS Z480, Bio-Rad CFX-96, CHKBiotech Q9600/Thunder series qPCR, SLAN96p, Molarray MA-6000, iNAT-POC and other real-time fluorescent PCR instruments, etc.

## 【SAMPLING】

Female cervical secretions: Women cannot urinate 2 hours before sampling, and sex is prohibited 24 hours before sampling. Place the cervical sampling brush in the cervix, gently rub the brush to rotate it clockwise for 4-5 times; slowly take out the cervical brush and put it into the sampling tube. Immerse the head of swab in the cell preservation solution and stir it for 5 seconds. Break the swab at the breaking point, cap and tighten the cap, and mark the number on the sample tube, and then send it to lab for HPV test.

Male genital tract secretions:Use a small sterile cotton swab to extend into the urethra for about 2 ~ 3cm, twist the

swab to collect secretions, place the cotton swab in the sample tube containing preservation solution (urination is prohibited within 2 hours before sampling).

The above mentioned cell preservation solution can be replaced by Virus Transportation Medium(Cat No.:VTM001), or Nucleic Acid release reagent(Cat No.:DL002) manufactured by CHKBiotech.

The sample can be used for testing immediately, or stored at 2-8°C for no more than 3 days, stored at -20°C for six months, and at -70°C for 2 years. The samples should be transported in curling to avoid repeated freezing and thawing.

## 【PROTOCOL】

1. Reagent preparation

a) Lyophilized in bottle version kit:Add **1mL** dissolving solution to the bottle to dissolve the lyophilized powder. Divide **20uL** of the dissolved reagent into each PCR reaction tube.

b) Lyophilized 8-well strip version kit: Add **20uL** dissolving solution to each tube well to resolve the PCR Mix.

c) Positive Control :Add **40uL** dissolving solution to one tube well of positive control to resolve it. Shock and centrifuge them at low speed.

The dissolved reagent and positive control can be temporarily stored at 4℃ for later use.

*Notes:When using the lyophilized in bottle version kit, after dissolving the reagent can be stored at -20℃ and repeated freeze -thaw should be less than 4 times.

2. DNA Extraction

Use the appropriate nucleic acid extraction kit and follow the extraction kit manual to extract nucleic acid from the specimen. Extraction reagent of magnetic beads(Cat No.:EX004) method and direct lysis method(Cat No.:DL002) of CHKBiotech are recommended. After DNA extraction, the extracted DNA should be added to the PCR reaction tube within 15 minutes, or transferred to a centrifuge tube and stored at -15℃~25℃.

3. Template Addition

Add 5uL negative control, 5uL positive control, and **5uL** nucleic acid extracted from each specimen to each PCR reaction tube. Vertex and centrifuge at low speed. Then, place them into the real-time PCR machine.

4. PCR Amplification

Recommended program setup

| | steps | temperature(℃) | time | cycles |
|---|---|---|---|---|
| 1 | UNG function | 50 | 2min | 1 |
| 2 | Pre-degeneration | 95 | 2min | 1 |
| 3 | Degeneration | 95 | 10s | 42 |
| 4 | Annealing and extension | 60 | 30s | |

*Note: Life Technologies real-time fluorescent quantitative PCR instrument specific parameter settings of the reaction program:

- "Select detectors" choose to add "Reporter" as FAM, HEX, ROX and CY5, and select "none" for "Quencher" and "Passive Reference";
- Amplification and fluorescence collection steps, choose to collect FAM, HEX, ROX and CY5 channel fluorescence in the step of "60℃";

5. Quality control

Negative and positive controls provide quality control should be tested for each run of test. The results are valid if all of the following conditions are met. Otherwise, the test is invalid. In this case, the instrument, reagent, amplification conditions, etc. should be checked for errors and the experiment should be repeated.

| Product quality control | Quality control requirements | | | |
|---|---|---|---|---|
| | FAM channel | HEX channel | ROX channel | CY5 channel |
| Positive control | Ct ≤ 32 | Ct ≤ 32 | Ct ≤ 32 | Ct ≤ 32 |
| Negative control | Undet | Undet or Ct>38 | Undet | Undet |

6. Result Analysis

| Channel | | | | Interpretation of results |
|---|---|---|---|---|
| FAM Channel | CY5 Channel | ROX Channel | HEX Channel | |
| Ct≤40 | Undet or Ct>40 | Undet or Ct>40 | Ct≤35 | HPV18 Positive |
| Undet or Ct>40 | Ct≤40 | Undet or Ct>40 | Ct≤35 | HPV16 Positive |
| Undet or Ct>40 | Undet or Ct>40 | Ct≤40 | Ct≤35 | Other HPV high-risk subtype Positive (among the 16 types) |
| Undet or Ct>40 | Undet or Ct>40 | Undet or Ct>40 | Ct≤35 | All the 18 HPV high-risk subetypes Negative |
| Any | Any | Any | Undet or Ct>35 | Retest*[1] |

*1 If the internal reference (HEX channel) Ct is Undet or Ct>35, the test result is invalid, and re-sampling and re-testing are required.

## 【CUT-OFF VALUE OR REFERENCE INTERVAL】

The positive judgment Cut-off value of HPV16,18 types and other HPV high-risk types are all Ct values ≤ 40.

## 【ASSAY EXPLAINATION】

1. Contamination of the laboratory environment and reagents, or cross-contamination during specimen processing may lead to false positive results.
2. When reagents are transported, stored, or manipulated incorrectly, they may even lead to false negative results, resulting in a decrease in the detection effect.

## 【ASSAY LIMITATIONS】

1. The positive result of the test kit does not indicate whether there is a virus in the body. It is recommended to use other confirmation methods at the same time.
2. This kit is used for the detection of HPV 18 high-risk subtypes and the classification of HPV high-risk types 16 and 18. This result is for clinical reference only, and the patient's clinical management should be considered in conjunction with the patient's symptoms/signs, medical history, other laboratory tests and treatment response, and should not be used as the only basis for clinical diagnosis and treatment;
3. A negative result cannot completely rule out HPV infection, and the target gene concentration in the sample below the detection limit can also cause a negative result;

## 【PERFORMANCE SPECIFICATIONS】

Accuracy: If the HPV corresponding type genotyping country or standardized enterprise positive reference product within the detection range of the test kit is tested, the test results are all positive.

Specificity: The test results are negative if the country or the standardized enterprise negative reference product is tested; the HPV corresponding type genotyping country or the standardized enterprise positive reference product in the detection range

of the test kit has no crossover. Response: The HPV corresponding type genotyping country or standardized enterprise positive reference product outside the detection kit detection range has no cross-reactivity for other HPV types.

Detection limitation:The detection limitation of HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82 types are all 500 copies/ml or 5 copies/Reaction.

Precision: Within the scope of the test kit, the HPV corresponding type genotyping country or standardized enterprise positive reference product and precision reference product were repeated 10 times, and the test results were consistent and all were positive for the corresponding type..

Analysis Specificity: no cross-reactivity with other related pathogens; normal concentration of hemoglobin, albumin, cervical mucus, vaginal contraceptives, feminine hygiene products, vaginal antifungal drugs, vaginal lubricants and other interfering substances are added to the simulated sample to test The result is no interference.

## 【ATTENTIONS】

1) This kit is only used for in vitro auxiliary diagnosis; the clinical diagnosis and treatment of patients should be comprehensively considered in conjunction with their symptoms/signs, medical history, other laboratory tests and treatment responses;
2) Please read the instructions of this kit carefully before the experiment, and strictly follow the operation steps;
3) Unreasonable sample collection, transfer, storage and processing procedures may lead to erroneous test results; if the sample processing process is not controlled, cross-contamination may occur, and false positive results may occur;
4) Steps such as sample processing must be carried out in a biological safety cabinet or other protective facilities.
5) Laboratory personnel must undergo professional training. The experiment process should be carried out in different areas (reagent preparation area, sample preparation area, amplification and product analysis area), and dedicated instruments and equipment should be used in each stage of the experimental operation, and supplies in each area and stage must not be used interchangeably; personnel flow and air flow in each area There should be strict requirements to avoid cross-contamination to the greatest extent.

## 【General Information】

Manufacturer: Shanghai Chuangkun Biotech Inc.

Address: Room 802, Building 1, No. 69 Yuanfeng Road, Baoshan District, Shanghai,PR.China

Contact information：+086-4000796006

E-Mail：admin@chkbio.com

**【European Anthorized Representative】**

EC REP

SUNGO Europe B.V.

Olympisch Stadion 24, 1076DE

Amsterdam,Netherlands

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