Human Papillomavirus DNA Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number
S3057E

Product Name
Human Papillomavirus DNA Diagnostic Kit (PCR-Fluorescence Probing)

Package Specification
48 tests/kit

Intended Use
The Human Papillomavirus DNA Diagnostic Kit (PCR-Fluorescence Probing) is intended for the qualitative detection of 15 kinds of high-risk human papillomavirus (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68) DNA in exfoliated cells from females' cervix, and also for the subtype identification of HPV16 and HPV18.

For in vitro diagnostic use only. For professional use only.

Summary
Clinical background: Human papillomavirus (HPV) is a kind of non-enveloped double-stranded circular DNA virus of small molecular weight. It infects and parasitizes epithelial cells of human reproductive organs and other organs. HPV falls into two types clinically, high-risk and low-risk HPV, according to the different degrees of pathogenicity or carcinogenic risk of different subtypes. Low-risk HPV mainly causes pathological changes such as exogeneity wart in anal skin, male external genitalia, female labia, urethral orifice and vagina lower segment, and low-grade cervical intraepithelial neoplasia. High-risk HPV can cause not only external genital warts, but more seriously, external genital cancer, cervical carcinoma and high-grade cervical intraepithelial neoplasia. The study shows that the persistent infection of HPV is the main cause of cervical carcinoma and precancerous lesion.1 Studies show the persistent infection of human papillomavirus is the main cause of cervical carcinoma and precancerous lesion. High-risk HPV DNA can be detected in 99.7% patients with cervical carcinoma. Because of the close relationship between high-risk HPV and cervical carcinoma, it is a significant screening method to test HPV for cervical carcinoma.2,3 Therapeutic effect of treating early precancerous lesions is much better than the therapeutic effect of treating cervical carcinoma. Therefore, the disease can be early diagnosed and treated through general survey, to reduce the morbidity and mortality of invasive carcinoma of cervix.

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 two popular kinds of medium-risk genotypes that are HPV53 and 66 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.4,5 Therefore, the diagnostic kit can be used for detection of the 15 kinds of target genotypes and also subtype identification of HPV16 and HPV18.

So far, it is difficult to test HPV by the use of conventional virus culture or serological technology. The main detection technology used in laboratory is molecular biological methods based on nucleic acid test. There are two main methods in this technology, one method based on target sequence amplification: type-specific PCR, mRNA amplification test, primer PCR, real-time (multiplex) PCR fluorescence technology, etc.,6,7 and the other method based on signal amplification technique: Amplification technique of liquid phase, morphology, enzyme digestion, etc.

Test Principle
By applying real-time fluorescence quantitative PCR technology, this diagnostic kit uses several pairs of specific primers and fluorescence probes which are designed to target E nucleic acid conserved sequences of the above mentioned 15 kinds of genotypes, accompanied with other ingredients in High-risk HPV-PCR mix, to achieve fast detection of HPV DNA, and detection of the 15 types of virus specimens and subtype identification of HPV16 and HPV18, through the changes of the four fluorescent signals (FAM, HEX/VIC, ROX, CY5).

The PCR detection system uses UNG enzyme + dUTP contamination-proof system to fully degrade possible PCR amplified products in order to avoid a false positive result.

The PCR detection system uses a positive control to monitor β-globin in human epidermal cells for the evaluation of amplification reaction of specimens and monitor the presence of PCR inhibitors, as well as to evaluate the nucleic acid extraction efficiency, in order to avoid a false negative result.

The FAM channel of this diagnostic kit is used to test HPV 18-DNA, HEX channel to test β-globin, ROX channel to test HPV 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68-DNA, and CY5 to test HPV 16-DNA.

Components of the Diagnostic Kit

No.	Reagent Name	Specification & Qty.	Main Ingredients
1	HPV-Enzyme Mix	192 μL/tube × 1 tube	DNA polymerase, Uracil-N-Glycosylase enzyme
2	HPV-PCR Mix	864 μL/tube × 2 tubes	Primer, probe, dNTPs, $MgCl_2$, PCR buffer
3	HPV-Positive Reference A (4.00E+07 copies/mL)	50 μL/tube × 1 tube	Cloning plasmid containing target gene fragment of HPV16, 18, 45 and β-globin
4	HPV-Positive Reference B (4.00E+06 copies/mL)	50 μL/tube × 1 tube	Cloning plasmid containing target gene fragment of HPV16, 18, 45 and β-globin
5	HPV-Positive Reference C (4.00E+05 copies/mL)	50 μL/tube × 1 tube	Cloning plasmid containing target gene fragment of HPV16, 18, 45 and β-globin
6	HPV-Positive Reference D (4.00E+04 copies/mL)	50 μL/tube × 1 tube	Cloning plasmid containing target gene fragment of HPV16, 18, 45 and β-globin
7	HPV-Negative Control	1000 μL/tube × 1 tube	Sterile saline
8	HPV-Positive Control	50 μL/tube × 1 tube	Cloning plasmid containing target gene fragment of HPV16, 18, 45 and β-globin

Note:
1. Do not mix or exchange components from different kit lots.
2. Please refer to the given value in the diagnostic kit for the specific concentration of HPV-Positive Reference A, B, C and D.
3. Self-prepared reagent: Cell preservation solution, Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number : S1006E) or Sample Release Reagent (Reference Number : S1013E) manufactured by Sansure Biotech Inc
4. Materials required but not provided: 1.5 mL DNase-free and RNase-free centrifuge tubes, 0.2 mL PCR reaction tubes, pipette tips (10 μL, 200 μL and 1000 μL tips with filters are preferred), desktop centrifuge, desktop vortex mixer, magnetic-bead separator, various models of pipette guns.

Storage
The reagents should be stored in sealed bottles at -20±5°C and protected from light. The shelf life of the kit is 12 months. Please see the manufacturing date and expiry date on the outer package.
1. The reagents keep stable at -20±5°C after opening. Once the reagents are used, the freeze/thaw cycles of the amplification reagent should not exceed five.
2. The reagents keep valid for 5 days during the shipment in a sealed foam box containing ice pack with the temperature lower than 20°C.

Compatible Instrument
The diagnostic kit is applicable to ABI7500, SLAN-96P instrument, MA-6000 PCR instrument and QuantGene 9600.

Specimen Requirements
1. Applicable specimen type: exfoliated cells from females' cervix.
2. Specimen collection:
2.1 Requirements before collection:
2.1.1 Specimen should not be collected during females' menstrual period.
2.1.2 The subjects should not have sex during the 48 hours before the collection.
2.1.3 The subjects should not have vaginal douching or have vaginal medication like contraceptive salve within 3 days before the collection.
2.2 Steps of using disposable sterile cervical sampler (containing cervical brush and cell preservation solution) to collect the samples are as follows.

For females who have suspected cervical infection, collect exfoliated cells from their cervix. Use a cotton swab to wipe the excessive secretions on the cervical opening. Put the cervical brush on the cervical opening and slightly rotate the brush clockwise for thee to four circles, then take out the cervical brush slowly and place it in a sample collection tube containing cell preservation solution. Break off the brush handle out of the tube, seal it and send for detection.

3. Specimen storage and delivery:
Specimens collected via the above-mentioned method can be used for immediate detection, or stored at room temperature for up to 24 hours, or at 2-8°C for less than 120 hours, or at -20±5°C for up to 7 months. Freeze/thaw cycles should not exceed seven. Specimens should be transported in a sealed frozen pitcher with ice or in a sealed foam box with ice. Keep the specimen at -70°C or lower for long term storage.

Test Method
1. Preparation of reagent (performed at "reagent preparation region")
1.1 Take out each component from the diagnostic kit and place them at room temperature. Allow the reagents to equilibrate at room temperature, and then vortex each of them respectively for future use.
1.2 According to quantity of test specimens, HPV-Negative Control, HPV-Positive Control and HPV-Positive References A-D, pipette appropriate quantity of HPV-PCR Mix and HPV-Enzyme Mix (HPV-PCR Mix 36 μL/test + HPV-Enzyme Mix 4 μL/test). Fully mix them to make a PCR-Mastermix and centrifuge them at 2000 rpm for 10 seconds. Keep it at 2-8°C for future use.

	1 sample	10 samples	24 samples	48 samples
HPV-PCR Mix (μL)	36	360	864	1728
HPV-Enzyme Mix	4	40	96	192

Note: The above configuration is just for your reference and to ensure enough volume of the PCR-Mastermix, more volume of the actual pipetting may be required.

2. Pretreatment of specimens (performed at "specimen processing region") (HPV-negative control should be processed and extracted together with specimens.)
Pipette 1 mL of specimen from the sample collection tube into a 1.5 mL centrifuge tube as the test specimen. Refer to the following steps to process the specimens.
Method 1: It is recommended to use Sample Release Reagent (Reference Number : S1013E) manufactured by Sansure Biotech Inc to extract the nucleic acid as per its instructions.
Method 2: It is recommended to use Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number : S1006E) manufactured by Sansure Biotech Inc to extract the nucleic acid as per their instructions.

3. Loading of Specimens (performed at "specimen processing region")
3.1 Processing of test specimens and HPV-Negative Control: respectively add 10 μL of the pretreated specimens and HPV-Negative Control into a PCR reaction tube (avoiding pipetting particulate matters like cell debris, etc.)
Processing of HPV-Positive Control and HPV-Positive References: respectively add 10 μL of HPV-Positive Control and HPV-Positive References A~D into a PCR reaction tube containing.
3.2 Add 40 μL of PCR-Mastermixes into each PCR reaction tube. Cover the tube lid (If there are bubbles, flick the tube to remove the bubbles), and centrifuge the tube at 2000 rpm for 10 seconds or lightly swing the tube until there no obvious liquid beads on the wall.

4. PCR Amplification (performed at "amplification and analysis region") (Refer to user manual of each instrument for the settings)
4.1 Place the PCR reaction tubes into the specimen wells of the amplification instrument. Input the settings of HPV-Negative Control, HPV-Positive Control, HPV-Positive References A-D and unknown specimens in the corresponding sequence, and input the sample name.
4.2 Selection of PCR test channel:
a) Select FAM channel to test HPV18-DNA.
b) Select HEX or VIC channel to test β-globin (Internal Control).
c) Select CY5 channel to test HPV16-DNA.
d) Select ROX channel to test HPV31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68-DNA.
4.3 Cycle parameters settings:

	Steps	Temperature	Time	No. of cycles
1	UNG enzyme reaction	50°C	2 min.	1
2	Predenaturation	94°C	5 min.	1
3	Denaturation	94°C	15 sec.	45
	Annealing, extension and fluorescence collection	57°C	30 sec.*	
4	Device cooling (optional)	25°C	10 sec.	1

***Note:** Due to the device ABI 7500's technical specifications, it cannot be set at 30 sec., but can be set at 31 sec. or 32 sec. When the settings are completed, save the settings and carry out the reaction procedure.

5. Result Analysis (Refer to user manual of each instrument for the settings)
Results will be saved automatically when reactions are completed. Adjust Start, End and Threshold values of Baseline of the graph according to analysis results (Users can adjust the values according to the actual situation. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "Analyze" to implement the analysis and make sure each parameter satisfy the requirements given in "6. Quality Control". Go to "Plate" window to record Ct value.

6. Quality Control
The test result is treated as valid if all the conditions in the table below are met for the same test. Otherwise the test result is treated as invalid and needs to be re-tested.

	HPV-Positive Control	HPV-Negative Control
Ct Value	24-30 at FAM, HEX/VIC, CY5 and ROX channel; Amplification curve of sample is like "S" shape.	No display at FAM, HEX/VIC, CY5 and ROX channel.

Positive Reference Values
Through the research on reference value (ROC curve method), the Ct positive reference value of the diagnostic kit for detecting the target gene is determined to be 39, and the Ct positive reference value for detecting the internal control is to be 40.

Explanation of Detection Results

1. Determination of negative or positive results

Conclusio	Ct value of sample at FAM, CY5 and ROX channel	Ct value of internal control at HEX/VIC channel
Positive	≤ 39	≤ 40
Others*	≤ 39	> 40 or N/A
Negative**	>39 or N/A	≤ 40

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Invalid***	>39 or N/A	> 40 or N/A

Note:* This suggests that there are no cervical epithelial cells in the specimen, but the patient has recently been exposed to HPV, so it can not be determined whether the patient is infected with HPV. It is suggested to collect the specimen again for detection.

** It includes that the concentration of high-risk HPV DNA is lower than the limit of detection or below the predetermined positive value.

*** This suggests an investigation should be carried out to find out the reasons when Ct value of the internal control is > 40 or N/A and retest it. (If repeated tests still produce invalid results, please contact Sansure Biotech at info@sansure.com.cn)

2. Subtype identification results

Determine the subtype identification result of high-risk human papillomavirus (15 types) according to the table below:

Detection result	Subtype identification
Sample is detected positive at CY5 channel.	HPV16 positive
Sample is detected positive at FAM channel.	HPV18 positive
Sample is detected positive at ROX channel.	Other high-risk HPV positive

Limitations of Detection Method

1. This diagnostic kit can be only used for the detection of the 15 types of high-risk human papillomavirus, which are HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68.
2. The detection results can be combined with cervix cytologic test results and other related medical examination results to comprehensively analyze the disease, but can not be used alone as the basis of patient management.
3. Possible reasons for a false negative result:
   3.1 Incorrect collection, delivery and processing, low viral titration may all result in a false negative result.
   3.2 Mutations in high-risk HPV target sequence or sequence change due to other causes may result in a false negative result.
   3.3 Incorrect storage of diagnostic kits may result in a false negative result.
   3.4 Other unproved interference or PCR inhibitors may also result in a false negative result.
4. Cross-contamination occurring in specimen processing may result in a false positive result.

Product Performance Index

1. Accuracy: When the kit is used to detect national genotype references of different genotypes or the standardized enterprise's work references, the results are all positive, and the subtype identifications of HPV16 and HPV18 are correct, and there are no cross-reactivities with other high-risk human papillomavirus.
2. Specificity: please see the cross-reactivities and its verified concentration level of verified other HPV genotypes and related pathogens in the table below:

Microorganism Name	Cross-reaction Concentration	Microorganism Name	Cross-reaction Concentration
Related Pathogens			
Chlamydia trachomatis	1.00E+06 CFU/mL	Ureaplasma urealyticum	1.00E+06 CFU/mL
Neisseria gonorrhoeae	1.00E+06 CFU/mL	Trichomonas vaginalis	1.00E+06 cells/mL
Mycoplasma hominis	1.00E+06 CFU/mL	Treponema pallidum	1.00E+06 copies/mL
Candida albicans	1.00E+06 CFU/mL	Herpes simplex virus type 2	5.00E+06 copies/mL
Human papillomovirus out of the detection scope of this diagnostic kit			
HPV6	1.00E+06 copies/mL	HPV11	1.00E+06 copies/mL
HPV26	1.00E+06 copies/mL	HPV40	1.00E+06 copies/mL
HPV42	1.00E+06 copies/mL	HPV43	1.00E+06 copies/mL
HPV44	1.00E+06 copies/mL	HPV54	1.00E+06 copies/mL
HPV61	1.00E+06 copies/mL	HPV67	1.00E+06 copies/mL
HPV69	1.00E+06 copies/mL	HPV70	1.00E+06 copies/mL
HPV71	1.00E+06 copies/mL	HPV72	1.00E+06 copies/mL
HPV73	1.00E+06 copies/mL	HPV81	1.00E+06 copies/mL
HPV82	1.00E+06 copies/mL	HPV83	1.00E+06 copies/mL

3. Precision: a precision study was carried out, which was tested twice per day on three lots of reagents by two different operators for 21 days. The test numbers of every sample is 84 (21 days, twice per day, two repetitions for every test). Within-run, between-run, between-lot, between-day, between-operator and %CV for total Ct value were less than 5%.
4. Limit of Detection: prepare a series of diluted samples to evaluate the lowest limit of detection for every different genotype of HPV DNA, and use 100% positive detection concentration level as the standard to determine the lowest limit of detection. The lowest limit of detection of every genotype is 1000 copies/mL.
5. Interfering substances: the concentration level and interference situations of the verified interfering substances are as below.

Interfering substances	Concentration level	Whether interference exists (yes or no)
hemoglobin	2 g/L	No
white blood cell	1.00E+07 cells/mL	No
cervical mucus	10%	No
contraceptive gel	0.5%	No
vaginal douche	0.5%	No
antifungal ointment, containing 2% Clotrimazole	0.5%	No
antifungal ointment, containing 2% Miconazole	0.5%	No
vaginal lubricant	10%	No

Precautions

1. The product can only be used for in vitro diagnosis. Please read the product manual carefully before operation.
2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory; laboratory personnel must receive professional training; test processes must be performed in separated regions; all consumables should be for single use only after sterilization; special instruments and devices should be used for every process; all lab devices used in different processes and regions should not be cross-used.
4. All specimens for detection should be handled as if infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of specimens and waste must meet relevant requirements outlined in local, state and national regulations.
5. Before use, all reagents must be fully thawed at room temperature and mixed thoroughly.
6. The product performance has been verified only on cervical epithelial cell samples and the samples collection and treatment methods (including specimen collection liquids etc.) of section **Specimen Requirements**. Other specimen types or sample collection, treatment methods can not guarantee the product performance.

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Symbols

Symbols	Meanings	Symbols	Meanings
IVD	In Vitro Diagnostic Medical Device		Date of Manufacture
	Use By		Consult Instructions for Use
	Temperature Limitation		Manufacturer
LOT	Lot Number	REF	Reference Number
Σ	Number of Tests	EC REP	Authorized representative in the European Community
	Any warnings and/or precautions to take	CE	This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

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