Nucleic Acid Detection Kit for 16&18 Human Papillomavirus (HPV) Genotypes (PCR fluorescence probe method)

REF 106-0064-01

Product Name
Nucleic Acid Detection Kit for 16&18 Human Papillomavirus (HPV) Genotypes (PCR fluorescence probe method)

Specification
12 reactions/kit

Intended Use
This product is an in vitro diagnostic reagents. It uses real-time multiplex fluorescent PCR technology, combined with the Automated Fully Enclosed qPCR Instrument and for the qualitative detection of high-risk HPV16 and HPV18 human papillomavirus (HPV) in cervical exfoliated cells, reproductive tract secretions and saliva in vitro Nucleic acid. The test results should be used for auxiliary diagnosis of HPV.
This kit uses the method of molecular biology for detection. It is required that the experimental operators should have received professional training in gene amplification or molecular biology method detection, have relevant experimental operation qualifications, and the laboratory should have reasonable biosafety facilities and protection procedures.

Test Principle
The Automated Fully Enclosed qPCR Instrument uses iCassette technology to automatically perform nucleic acid extraction, nucleic acid amplification, data reading and result analysis. The instrument includes two parts of nucleic acid extraction and multiplex fluorescent PCR detection. At the same time, the built-in QR code scanner can automatically identify the execution process corresponding to this kit. Execute the whole process of extraction and PCR of this kit, display the results and analyze the results through the uniquely designed software. This kit contains 12 disposable HPV iCassettees. The reagents stored in the iCassette include nucleic acid extraction reagents and HPV PCR reagents. It is suitable for the Automated Fully Enclosed qPCR Instrument. Since the Automated Fully Enclosed qPCR Instrument is fully enclosed and automatically performs nucleic acid extraction and PCR reactions, it reduces direct cross-contamination of samples. For a complete description of the instrument, please refer to the corresponding instrument user manual.
This kit includes nucleic acid extraction reagents and HPV PCR reagents. Before the PCR reaction starts, the Automated Fully Enclosed qPCR Instrument first tests the fluorescent signal of the probe, which is used to monitor whether the PCR reagent is fully reconstituted, whether the PCR reaction tube is added to the sample, and monitor the integrity and stability of the probe condition.
This kit uses the L1 variation region of the HPV genome as the detection target region, respectively designs specific primers and fluorescent probes, performs PCR amplification in a PCR reaction tube, and simultaneously detects HPV16 (TEXAS RED marker), HPV18 (FAM marker) and internal standard (CY5 marker). Therefore, it is possible to type HPV16 and HPV18 human papillomaviruses in one iCassette, and monitor the extraction and amplification process of the kit through the internal standard.

Components

Kit Components			Content	Quantity
HPV 16&18 iCassette (12 pcs)	Nucleic acid extraction reagent	Magnetic beads	Magnetic Microspheres	13μL/iCassette
		HPV lyophilization A	Proteinase K	1 pcs/iCassette
		Virus lysate solution	Guanidine isothiocyanate	450 μL/iCassette
		Virus binding solution	Guanidine isothiocyanate	175 μL/iCassette
		Virus washing solution	Sodium chloride	900 μL/iCassette
		Virus eluent	Tris-HCl	100 μL/iCassette
		HPV cosolvent solution	PCR Buffer、$MgCl_2$	35μL/iCassette
	HPV PCR reagent	HPV16&18 reaction tube	Specific primer probe (HPV16, 18, internal control), dNTP, enzyme	1 pc/tube
Control	HPV positive control		HPV18 type plasmid	1tube(200μL)
	HPV negative control		β-actin plasmid	1tube(200μL)

Note: Components in kits with different batch numbers are not interchangeable.

Storage condition and Shelf life
1. The kit can be stored at 2-8℃ and the shelf life is 9 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette cover before adding the sample. If you open the iCassette cover, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument: Galaxy Nano, Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Nano, Galaxy Lite or Galaxy Pro.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Vortex instrument.
- Pipettes.
- Sterile pipette tips.

Sample Requirements
1. Applicable sample types: cervical exfoliated cells, reproductive tract secretions and saliva.
2. Sample collection
2.1 Sampling equipment requirements
2.1.1 Swab: The head used to sample should be the synthetic fiber (eg, polyester fiber), and use aluminum or plastic as a rod.
2.1.2 Sampling tube: Contains 3mL physiological saline or sampling liquid (containing protein stabilizers, antibiotics, buffer to prevent bacteria and fungal growth).
2.2 Cervical exfoliated cells: Before sampling, gently wipe the excessive secretions with the cervical mouth with a cotton swab, and place the cervical collection in the cervical mouth to gently rotate 3-5 laps to collect the cervical falling cells, put it into the sampling pipe, and put it into the sampling tube. Concerned inspection.
2.3 Reproductive tract secretions.
2.3.1 Female reproductive tract secretions: First use aseptic physiological saline cotton balls to clean the urethra, then insert an urethra with a sterile cotton swab to 2cm, and rotate the cotton swab to collect the urethral secretion after 5 seconds. , put the swab into the sampling tube and seal it for inspection (forbidden to urinate 2 hours before the collection of secretions).
2.3.2 Men's reproductive tract secretions: Use a small sterile cotton swab to reach into the urethra about 2 ~ 4 cm, and rotate to obtain the reproductive tract secretion. Put the cotton swabs in the sampling tube and seize the inspection (forbidden to urinate 2 hours before the collection of secretions).
2.4 Saliva:
2.4.1 30 minutes before collecting saliva samples, do not eat, drink water or smoke, etc., and rinse your mouth with water.
2.4.2 Before collecting saliva, relax the cheeks and gently massage the cheeks with your fingers for 15-30 seconds to produce saliva. Collect 2mL saliva according to the saliva collector instructions. After collecting saliva, tighten the tube cover with the collection tube. Invert the collection tube several times to fully mix the saliva and preservation solution, and put the used collection funnel into a recyclable trash can. For the collector that does not contain the savage, the tube cover is tightened directly after the collection, and place used collection funnels in recyclable bins. Mix the saliva and the saving solution at a ratio of 1: 1.
3. Sample storage and transportation
All collected cervical falling cells and reproductive tract secretions should be packaged immediately when collecting in the hospital. One in one portion, one of which is stored separately to prepare for a review. The sample should be stored at 2-8℃, for detecting within 12 hours, or stored -20℃ or below as soon as possible, but should not exceed 1 month.
4. Principles of Biosafety Protection：
All operations should comply with local relevant laws and regulations.

Test Method and Operation
1. Prepares HPV 16&18 iCassette
1.1Processing samples or control materials in the samples preparation room,first vortex the collection tube or controls for 10-15 seconds.
1.2 Open the package of HPV 16&18 lyophilization, observe whether the HPV 16&18 lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the HPV 16&18 lyophilization, and make sure the PCR tube is screwed up.
1.3 Open the lid of the iCassette, and pipette 200 μl of sample or controls to the HPV 16&18 iCassette sample compartment as shown in Figure 1. slowly, close the lid tightly.(Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)
1.4 Place the iCassette into the instrument.

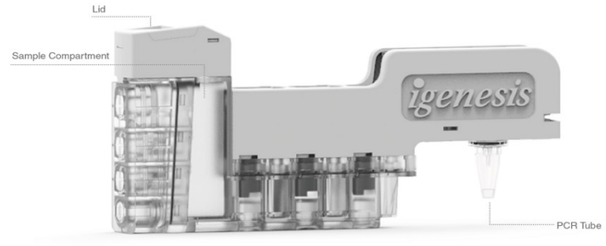

Figure 1 HPV 16&18 iCassette (Lateral View)

2. Test Operation
2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.
2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.
2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.
2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the corresponding channel position of the control software while loading the iCassette)
2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.
2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.
2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.
2.8 After the amplification is completed, the compartment door will open automatically.
For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis
After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
Test results of positive control: FAM channel is positive, CY5 channel is positive, others are negative;
Test results of negative control: CY5 channel is positive, others are negative;
Internal standard control: CY5 channel is positive (there is a specific competition in the internal standard and samples. When the sample is detected as positive, the internal standard may be detected as negative. When the clinical sample is negative, the internal standard must be positive, otherwise the sampling failure or the extraction failed)..
The above requirements must be met at the same time in the same test. Otherwise, the test is invalid and needs to be repeated.

Reference Interval
Based on the analysis of clinical sample test results, using the ROC curve method, the final determined Ct positive threshold value for this reagent kit is 30.

Interpretation of Test Results

Channels	CY5 Channel	TEXAS RED Channel	FAM Channel
HPV16&18 Reaction Tube	Internal Control	16 Type	18 Type

The sample to be tested will be determined according to the standards in the table below:

FAM Channel	TEXAS RED Channel	CY5 Channel	Results
×	√	√/×	HPV16 positive.(Figure 2)
√	×	√/×	HPV18 positive (Figure 3)
×	×	√	Negative
√	×	√	Positive control
×	×	√	Negative control

Note: "√" means that the result "has an obvious logarithmic amplification curve", and Ct≤30; "×" indicates the result "no logarithmic amplification curve", and Ct >30."√/×" indicates that the result "may be positive or negative".The CY5 channel is the internal standard channel. Because there are specific competition with the sample, when the sample is detected as positive, it may be detected as negative.

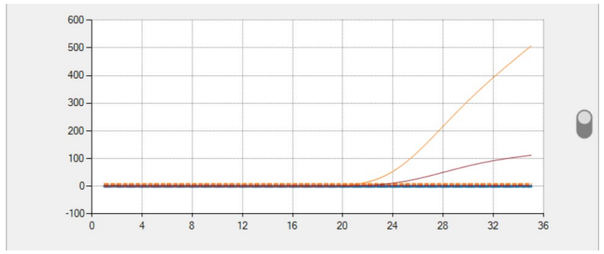

Figure 2. HPV16 Type Positive

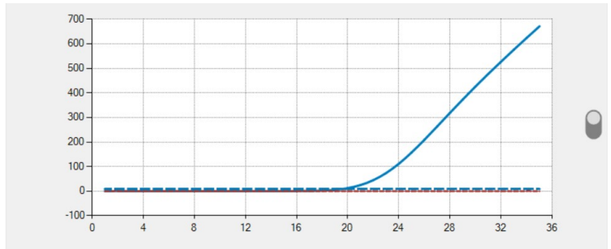

Figure 3. HPV 18 Type Positive

Limitations of Test Method

1.The test results should be comprehensively analyzed in conjunction with the clinical symptoms of patients and other related medical examination results, and it should not be used as a basis for patient management.
2.Unreasonable sample collection, transportation and processing, low virus titer in the sample, and improper experimental operations and experimental environment may cause false negative results.
3.The variation of the target sequence of HPV or the sequence changes caused by other reasons may cause false negative results.
4.Due to the inconsistent positive rate of sample samples at different stages of different diseases, the most suitable sample type and the optimal sampling time after infection may not be confirmed. Therefore, the possibility of repeating samples in the same patient will reduce the false negative results.

Product Performance Index]

1. Analysis Sensitivity
The HPV16 type and HPV18 type samples in "the national positive reference of Hmuan Papillomavirus L1 genetic types" used as the samples to be tested are the sensitivity samples S1, S2 respectively. The detection sensitivity is 400copies/mL. Both are positive and the compliance rate is 100 %.
2. Accuracy
The HPV16 type and HPV18 type samples in "the national positive reference of Human Hmuan Papillomavirus L1 genetic types" used as the samples to be tested are the accuracy samples P1, P2 respectively. The concentration is 1×10^3copies/mL. Both are positive and the compliance rate is 100 %.
3. Analysis Specificity
Specific detection samples: Sample 1-16 comes from high-risk HPV26, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV56, HPV59 HPV66, HPV73 and HPV82 and low-risk HPV6, HPV11, HPV40, HPV42, HPV43, HPV44, HPV54, HPV61, HPV67, HPV69, HPV70, HPV71,HPV72, HPV81, HPV83, CP8304, and other pathogens that are similar to species or cause similar symptoms such as Cytomegalovirus, Herpes simplex virus 2, Syphilis, M.urealyticum, M.hominis, Neisseria gonorrhoeae, Chlamydia trachomatis, Candida albicans, Vaginal Trichomonas. The samples are all confirmed by the corresponding kit as the corresponding pathogen positive, with a total of 41 copies.

No.	Type	Concentration	No.	Type	Concentration
1	HPV26	1×10^4 copies/mL	22	HPV44	1×10^4 copies/mL
2	HPV31	1×10^4 copies/mL	23	HPV54	1×10^4 copies/mL
3	HPV33	1×10^4 copies/mL	24	HPV61	1×10^4 copies/mL
4	HPV35	1×10^4 copies/mL	25	HPV67	1×10^4 copies/mL
5	HPV39	1×10^4 copies/mL	26	HPV69	1×10^4 copies/mL
6	HPV45	1×10^4 copies/mL	27	HPV70	1×10^4 copies/mL
7	HPV51	1×10^4 copies/mL	28	HPV71	1×10^4 copies/mL
8	HPV52	1×10^4 copies/mL	29	HPV72	1×10^4 copies/mL
9	HPV53	1×10^4 copies/mL	30	HPV81	1×10^4 copies/mL
10	HPV56	1×10^4 copies/mL	31	HPV83	1×10^4 copies/mL
11	HPV58	1×10^4 copies/mL	32	CP8304	1×10^4 copies/mL
12	HPV68	1×10^4 copies/mL	33	Cytomegalovirus	1×10^6 copies/mL
13	HPV59	1×10^4 copies/mL	34	Herpes simplex virus2	1×10^6 copies/mL
14	HPV66	1×10^4 copies/mL	35	Syphilis	1×10^6 copies/mL
15	HPV73	1×10^4 copies/mL	36	M.urealyticum	1×10^6 copies/mL
16	HPV82	1×10^4 copies/mL	37	M.hominis	1×10^6 copies/mL
17	HPV6	1×10^4 copies/mL	38	Neisseria gonorrhoeae	1×10^6 copies/mL
18	HPV11	1×10^4 copies/mL	39	Chlamydia trachomatis	1×10^6 copies/mL
19	HPV40	1×10^4 copies/mL	40	Candida albicans	1×10^6 copies/mL
20	HPV42	1×10^4 copies/mL	41	Vaginal Trichomonas	1×10^6 copies/mL
21	HPV43	1×10^4 copies/mL			

All the above specific samples were negative by this kit test, which was in line with the expected setting of the kit.
4. Precision:
The HPV18 type sample in "the national positive reference of Human Hmuan Papillomavirus L1 genetic types" with the concentration 1×10^3 copies/mL used as the samples R1 to be tested, after testing, the intra-assay precision coefficient of variation CV≤5%.

Precautions]

1.The kit should be used within the validity period.
2. Do not open the iCassette lid before use. If opened, use it within 30 minutes.
3. Each iCassette is a single-used, please do not reuse it.
4. If the iCassette is oscillated after adding the sample, do not use the iCassette.
5. Prevent sample contamination; During the experiment, the medical staff must wear work clothes, disposable gloves, and use self-discharging pipettes.
6. Clinical laboratories should strictly follow the management standards in the local related regulations for molecular biology laboratories and clinical gene amplification laboratories.
7. This kit is intended for in vitro diagnosis only.

References

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Instruction Version

Version: A/0
Date of Issue:May,2022

Symbols

The following symbols may appear on the product labeling:

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
REF	Catalogue number	CE	CE marking of conformity
EC REP	Authorized representative in the European Community		

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