Nucleic Acid Detection Kit for 18 High-risk and Low-risk 6&11 Human Papillomavirus (HPV) Genotypes (PCR-Fluorescence)

REF 106-0062-01

Product Name
Nucleic Acid Detection Kit for 18 High-risk and Low-risk 6&11 Human Papillomavirus (HPV) Genotypes (PCR-Fluorescence)

Specification
6 reactions/kit

Intended Use
This kit uses real-time multiplex fluorescent PCR technology, combined with an Automated Fully Enclosed qPCR Instrument, for in vitro qualitative detection of 18 high-risk types (HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV68, HPV59, HPV66, HPV73 and HPV82) and 2 low-risk (HPV6 and HPV11) nucleic acids in human cervical exfoliated cells or reproductive tract secretions samples. The test results can be used for HPV auxiliary diagnosis.
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, amplification, data reading and result analysis. The instrument includes two parts of nucleic acid extraction and multiplex fluorescent PCR. At the same time, the built-in QR code scanner can automatically identify the execution process corresponding to this icassette. Execute the whole process of extraction and amplification of this kit, display the results and analyze the results through the uniquely designed software. This kit contains 6 disposable HPV20 typing icassette. The reagents stored in the icassette include nucleic acid extraction reagents and PCR reaction reagents, which are suitable for Automated Fully Enclosed qPCR Instrument. Since instrument is fully enclosed and automatically performs nucleic acid extraction and PCR reactions, direct cross-contamination of samples is reduced. For a complete description of the instrument, please refer to the corresponding instrument user manual.
This kit includes nucleic acid extraction reagents and PCR reaction reagents. Before the PCR reaction starts, the Automated Fully Enclosed qPCR Instrument will first test the fluorescent signal of the probe to monitor whether the PCR reagent is fully reconstituted, whether the sample is added to the PCR reaction tube, and test the integrity and stability of the probe.
This kit uses the L1 variation region of the HPV genome as the detection target region, respectively designs specific primers and fluorescent probes, and performs PCR amplification in 3 PCR reaction tubes, including HPV6 type (AMCA marker) and HPV16 type (TEX marker) , HPV39 (TAMARA marker), HPV51 (FAM marker), HPV52 (CY5.5 marker), HPV58 (HEX marker) and HPV73 (CY5 marker) were detected in HPV reaction tube 1; HPV11 (AMCA marker), HPV45 (HEX marker), HPV53 (TEX marker), HPV56 (FAM marker), HPV59 (CY5 marker), HPV66 (CY5.5 marker), HPV82 (TAMARA marker) in HPV reaction tube 2; HPV18 (FAM marker), HPV26 (HEX marker), HPV31 (CY5 marker), HPV33 (CY5.5 marker), HPV35 (AMCA marker), HPV68 (TEX marker) and internal standard (TAMARA marker) was detected in HPV reaction tube 3. Thus, 18 high-risk and 2 low-risk HPV types (HPV6 and HPV11) can be fully typed in one iCassette.

Components

Kit Components			Content	Quantity
HPV 20 genotypes iCassette	Nucleic acid extraction reagent	Magnetic beads	Magnetic Microspheres	11 μL/iCassette
		HPV lyophilization A	Proteinase K	1 pc/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	90 μL/iCassette
		HPV cosolvent solution	PCR Buffer, $MgCl_2$	35 μL/iCassette
	HPV PCR reagent	HPV reaction tube 1	Specific primer probe (HPV6, 16, 39, 51, 52, 58), dNTP, enzyme	1 pc/tube
		HPV reaction tube 2	Specific primer probe (HPV11, 45, 56, 59, 66, 82), dNTP, enzyme	1 pc/tube
		HPV reaction tube 3	Specific primer probe (HPV18, 26, 31, 33, 35, 68, Internal standard), 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 lid before adding the sample. If you open the iCassette lid, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument: Galaxy Lite,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 and reproductive tract secretions.
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).
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 ° C or below as soon as possible.
4.Principles of Biosafety Protection
All operations should comply with local relevant laws and regulations.

Test Method and Operation
1. Prepare HPV 20 genotypes 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 reaction tube 1/HPV reaction tube 2/HPV reaction tube 3, observe whether the freeze-dried balls in the HPV reaction tube are complete, and replace these 3 HPV reaction tubes with the empty PCR threaded tubes at the corresponding positions on the iCassette as shown in Figure 1 (such as reaction Tube 1 is placed in the "1" position), and make sure that the HPV reaction tube is tightened.
1.3 Open the lid of the iCassette, and pipette 200 μl of sample or controls to the HPV 20 genotypes 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.

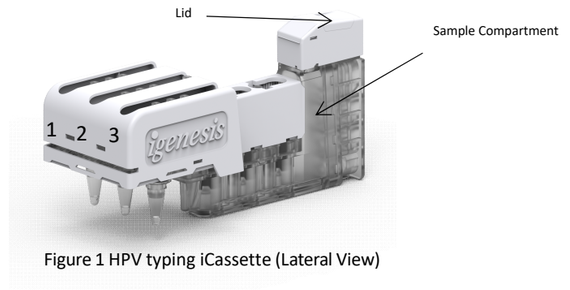

Figure 1 HPV typing iCassette (Lateral View)

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: All channels of reaction tubes 1 and 2 are negative, the FAM channel of reaction tube 3 is positive, and the other channels are negative;
Test results of negative control: All channels of reaction tubes 1 and 2 are negative, the TAMARA channel of reaction tube 3 is positive, and the other channels are negative;
Internal standard control: All channels of reaction tubes 1 and 2 are negative, the TAMARA channel of reaction tube 3 is positive, and the other channels are negative
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	Cy5.5 Channel	TEX Channel	FAM Channel	HEX Channel	AMCA Channel	TAMARA Channel
Reaction Tube 1	HPV 73 Type	HPV 52 Type	HPV 16 Type	HPV 51 Type	HPV 58 Type	HPV 6 Type	HPV 39 Type
Reaction Tube 2	HPV 59 Type	HPV 66 Type	HPV 53 Type	HPV 56 Type	HPV 45 Type	HPV 11 Type	HPV 82 Type
Reaction Tube 3	HPV 31 Type	HPV 33 Type	HPV 68 Type	HPV 18 Type	HPV 26 Type	HPV 35 Type	Internal Standard

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

No.	FAM Channel	HEX Channel	TAMRA Channel	TEX Channel	Cy5 Channel	Cy5-5 Channel	AMCA Channel	Results
PCR Reaction Tube 1	√	×	×	×	×	×	×	HPV51 Positive
	×	√	×	×	×	×	×	HPV58 Positive
	×	×	√	×	×	×	×	HPV39 Positive
	×	×	×	√	×	×	×	HPV16 Positive(Figure 2)
	×	×	×	×	√	×	×	HPV73 Positive
	×	×	×	×	×	√	×	HPV52 Positive
	×	×	×	×	×	×	√	HPV6 Positive (Figure 3)
No.	**FAM Channel**	**HEX Channel**	**TAMRA Channel**	**TEX Channel**	**Cy5 Channel**	**Cy5-5 Channel**	**AMCA Channel**	**Results**
PCR Reaction Tube 2	√	×	×	×	×	×	×	HPV56 Positive
	×	√	×	×	×	×	×	HPV45 Positive
	×	×	√	×	×	×	×	HPV82 Positive
	×	×	×	√	×	×	×	HPV53 Positive
	×	×	×	×	√	×	×	HPV59 Positive
	×	×	×	×	×	√	×	HPV66 Positive
	×	×	×	×	×	×	√	HPV11 Positive (Figure 4)
No.	**FAM Channel**	**HEX Channel**	**TAMRA Channel**	**TEX Channel**	**Cy5 Channel**	**Cy5-5 Channel**	**AMCA Channel**	**Results**
PCR Reaction Tube 3	√	×	×/√	×	×	×	×	HPV18 Positive (Figure 5)
	×	√	×/√	×	×	×	×	HPV26 Positive
	×	×	×/√	√	×	×	×	HPV68 Positive
	×	×	×/√	×	√	×	×	HPV31 Positive
	×	×	×/√	×	×	√	×	HPV33 Positive
	×	×	×/√	×	×	×	√	HPV35 Positive
	×	×	√	×	×	×	×	Negative

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 TAMARA channel of the reaction tube 3 is an internal standard channel. Because there are specific competition with the sample, when the sample is detected as positive, it may be detected as negative.

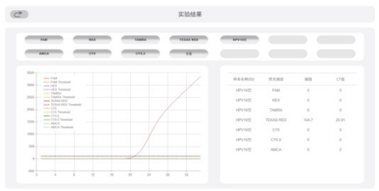

Figure 2.HPV16 Type Positive

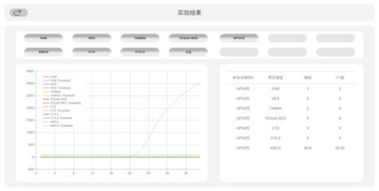

Figure 3.HPV 6 Type Positive

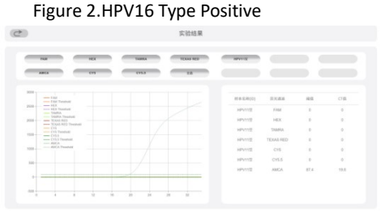

Figure 4.HPV11 Type Positive

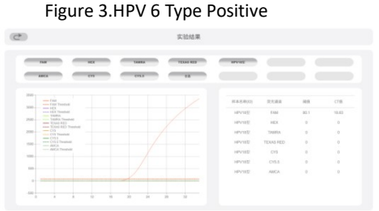

Figure 5.HPV 18 Type Positive

Limitations of Test Method

1. The test results of this reagent 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 HPV6、HPV11、HPV16、HPV18、HPV26、HPV31、HPV33、HPV35、HPV39、HPV45、HPV51、HPV52、HPV53、HPV56、HPV58、HPV59、HPV66、HPV68、HPV73、HPV82 samples in "the national positive reference of Hmuan Papillomavirus L1 genetic types" used as the samples to be tested are the sensitivity samples S1~S20 respectively. The detection sensitivity is 400 copies/mL. Both are positive and the compliance rate is 100 %.

2. Accuracy

The HPV6、HPV11、HPV16、HPV18、HPV26、HPV31、HPV33、HPV35、HPV39、HPV45、HPV51、HPV52、HPV53、HPV56、HPV58、HPV59、HPV66、HPV68、HPV73、HPV82 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~P20 respectively. The concentration is 1 × 103 copies/mL. Both are positive and the compliance rate is 100 %.

3. Analysis specificity

Specific detection samples: Sample 1-16 are 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 23.

No.	Type	Concentration	No.	Type	Concentration
1	HPV40	1×10^4 copies/mL	13	HPV83	1×10^4 copies/mL
2	HPV42	1×10^4 copies/mL	14	CP8304	1×10^4 copies/mL
3	HPV43	1×10^4 copies/mL	15	Cytomegalovirus	1×10^6 copies/mL
4	HPV44	1×10^4 copies/mL	16	Herpes simplex virus 2	1×10^6 copies/mL
5	HPV54	1×10^4 copies/mL	17	Syphilis	1×10^6 copies/mL
6	HPV61	1×10^4 copies/mL	18	M.urealyticum	1×10^6 copies/mL
7	HPV67	1×10^4 copies/mL	19	M.hominis	1×10^6 copies/mL
8	HPV69	1×10^4 copies/mL	20	Neisseria gonorrhoeae	1×10^6 copies/mL
9	HPV70	1×10^4 copies/mL	21	Chlamydia trachomatis	1×10^6 copies/mL
10	HPV71	1×10^4 copies/mL	22	Candida albicans	1×10^6 copies/mL
11	HPV72	1×10^4 copies/mL	23	Vaginal Trichomonas	1×10^6 copies/mL
12	HPV81	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 vibrated after adding the sample and leaks, please do not continue to use it.
5. Prevent sample contamination; During the experiment, the medical staff must wear work clothes, disposable gloves, and use self-discharging pipettes.
6. In order to avoid any potential biological hazards in the sample, the test sample should be treated as infectious material and avoid contact with skin and mucous membranes; Sample handling and processing must comply with relevant regulations.
7. This kit is intended for in vitro diagnosis only.

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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