Diagnostic Kit for detecting of 16 high-risk and genotyping of 16&18 human papillomavirus (HPV) (PCR fluorescence probe method)

REF 106-0066-01

Product Name
Diagnostic Kit for detecting of 16 high-risk and genotyping of 16&18 human papillomavirus (HPV) (PCR fluorescence probe method)

Specification
12 reactions/kit.

Intended Use
The kit uses real-time multiple fluorescent PCR technology that combined with an Automated Fully Enclosed qPCR Instrument. It is used for the in vitro qualitative detection of high-risk HPV16, HPV18 and other 16 high-risk (HPV26, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV68, HPV59, HPV66, HPV73 and HPV82) human papillomavirus (HPV) nucleic acids in cervical exfoliated cells or reproductive tract secretions. The test results can be used for HPV auxiliary diagnosis.
The kit uses molecular biology methods to detect. The operators should have received professional training in gene amplification or molecular biological methods and have relevant experimental operating qualifications. The laboratory should have reasonable biological safety facilities and protective procedures.

Test Principle
The Automated Fully Enclosed qPCR Instrument uses iCassette technology to automatically perform nucleic acid extraction, amplification, reading data and result analysis. The instrument includes two parts: nucleic acid extraction and multiple fluorescence PCR. At the same time, a built-in QR code scanner can automatically identify the execution process corresponding to this kit. The uniquely designed software is used to perform the extraction of the kit and the whole process of PCR, display results and analysis results. The kit contains 12 disposable HPV iCassette. The reagents stored in the iCassette include nucleic acid extraction reagents and HPV PCR reagents which are suitable for Automated Fully Enclosed qPCR Instrument. Since the fluorescent PCR integrated machine 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 instruction for use of the corresponding instrument.
The kit includes nucleic acid extraction reagents and HPV PCR reagents. Before the start of the PCR reaction, the Automated Fully Enclosed qPCR Instrument will test the fluorescent signal of the probe to monitor whether the PCR reagents are fully reconstituted whether the sample is added to the PCR reaction tube, and monitor the integrity and stability of the probe condition.
This kit uses the L1 variant region of the HPV genome as the detection target region to design specific primers and fluorescent probes respectively. The PCR amplification in a PCR reaction tube can simultaneously detect HPV16 (TEXAS RED labelled), HPV18 (FAM labelled), other 16 high-risk types (31,33,45,51,52,56,58 HEX labelled; 39,59,68,73 CY5 labelled; 26,35,53,66,82 CY5.5 labelled) and internal control (AMCA labelled). Therefore, it is possible to type HPV16 and HPV18, and detect other 16 high-risk types of human papillomavirus without typing at the same time in a iCassette, and monitor the extraction and amplification process of the kit through internal control.

Components

Kit Components			Content	Quantity
HPV 2+16 iCassette (12 pcs)	Nucleic acid extraction reagent	Magnetic beads	Magnetic microspheres	11μL/ iCassette
		HPV lyophilization A	Proteinase K	1pc/ iCassette
		Virus lysate solution	Guanidine Isothiocyanate	450μL/ iCassette
		Virus binding Solution	Guanidine Isothiocyanate	175μL/ iCassette
		Virus washing solution	Sodium chloride	900μL/ iCassette
		Virus eluent	Tris-HCl	90μL/ iCassette
		HPV cosolvent solution	PCR Buffer, $MgCl_2$	35μL/ iCassette
	HPV PCR reagent	HPV 2+16 reaction tube	Specific primer probe, internal standard primer probe, dNTP, enzyme	1 pc/tube
Control	HPV positive control		HPV18 plasmid	1tube(200μL)
	HPV negative control		plasmid fragment of β globulin	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.Sample types: cervical exfoliated cells and genital secretions.
2.Sample collection
2.1 Cervical exfoliated cells: Before sampling, medical staff should use cotton swabs to gently wipe the excessive secretions of the cervix, place the cervix collection brush on the cervix and gently rotate 3-5 times to collect the exfoliated cells from the cervix and place them in a sterile cell preservation solution. The preservation tube is sealed and submitted for inspection.
2.2 Genital secretions
2.2.1 Female genital tract secretions: The medical staff first clean the vagina with a cotton ball of sterile normal saline and then insert a sterile cotton swab into the vagina for about 2 cm. After stopping for 5 seconds, turn the cotton swab to collect urethral secretions. Put the cotton swab into a sterile preservation tube with cell preservation solution and submit it for testing (no urination 2 hours before collection of secretions).
2.2.2 Male genital tract secretions: The medical staff uses a small sterile cotton swab to extend into the urethra for about 2 to 4 cm and rotate it to obtain genital secretions. Put the cotton swab into a sterile preservation tube with cell preservation solution and submit it for testing (no urination 2 hours before collection of secretions).
3.Sample storage and transportation
All collected samples of cervical exfoliated cells and genital tract secretions should be divided into duplicates immediately when collected in the hospital and one of them should be kept separately for review. The sample should be used for testing within 7 days under the storage condition of 2-8℃. If the test cannot be carried out within 7 days, it should be placed at -20℃.
4.Principles of Biosafety Protection
All operations should comply with local relevant laws and regulations.

Test Method and Operation
1. Prepare the HPV 2+16 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 2+16 lyophilization, observe whether the HPV 2+16 lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the HPV 2+16 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 2+16 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.

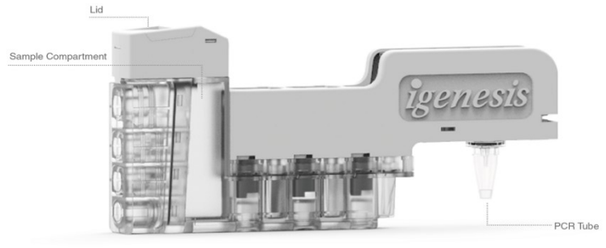

Figure 1 HPV 2+16 iCassette (Lateral View)

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
The test result for the positive control: The FAM channel is positive, AMCA channel is positive and other channels are negative.
The test result for the negative control: The AMCA channel is positive and other channels are negative.
Internal control: The AMCA channel is positive (the internal control is specifically competing with the sample. When the sample is positive, the internal control may be tested negative. When the sample is negative, the internal control must be positive. Otherwise it may be a sampling failure or test failure).
The above requirements must be met at the same time in the same experiment. Otherwise, the experiment 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	AMCA Channel	TEXAS RED Channel	FAM Channel	HEX/CY5/CY5.5 Channel
HPV 2+16 reaction tube	Internal control	HPV16	HPV18	Other 16 high-risk

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

FAM Channel	TEXAS RED Channel	HEX/CY5/CY 5.5 Channel	AMCA Channel	Result
×	√	×	√/×	HPV16 Positive (Figure 2)
√	×	×	√/×	HPV18 Positive (Figure 3)
×	×	√	√/×	Other high-risk positive.
×	×	×	√	Negative
√	×	×	√	Positive control
×	×	×	√	Negative control

Note: "√" means that the result "has an obvious logarithmic amplification curve, and value of Ct is less than or equal to 30". "×" means the result is "no logarithmic amplification curve or value of Ct is more than 30". "√/×" means that the result "may be positive or negative". The AMCA channel is an internal standard channel. Due to specific competition with the sample, when the test sample is positive, it may be tested negative.

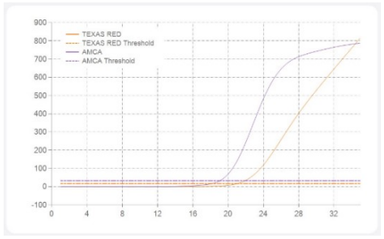

Figure 2 HPV16 Positive

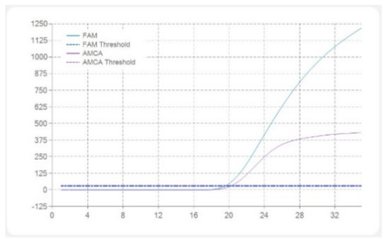

Figure 3 HPV18 Positive

Limitations of Test Method

1.The test results of this reagent should be combined with the patient's clinical symptoms and other relevant medical examination results for comprehensive analysis and it should not be used as a basis for patient management alone.
2.Unreasonable sample collection, transportation and processing, as well as improper experimental operation and experimental environment may lead to false negative or false positive results.
3.Variations in the HPV target sequence to be tested or sequence changes caused by other reasons may cause false negative results.
4.Since the positive rates of samples from different stages of the disease are inconsistent, the most suitable type of sample for detection and the best sampling time after infection may not be confirmed. Therefore, collecting samples from the same patient multiple times will reduce the possibility of false negative results.

Product Performance Index

1.Analysis Sensitivity
The samples of HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV68, HPV59, HPV66, HPV73 and HPV82, are prepared according to the preparation method in "National Reference Materials for L1 gene of Human Papillomavirus genotyping" as the samples to be tested and they are used as sensitivity samples S1-S18. The concentrations are shown in the following table:

No.	Type	Concentration		No.	Type	Concentration
S1	HPV16	400 copies/mL		S10	HPV52	400 copies/mL
S2	HPV18	400 copies/mL		S11	HPV53	400 copies/mL
S3	HPV26	400 copies/mL		S12	HPV56	400 copies/mL
S4	HPV31	400 copies/mL		S13	HPV58	400 copies/mL
S5	HPV33	400 copies/mL		S14	HPV68	400 copies/mL
S6	HPV35	400 copies/mL		S15	HPV59	400 copies/mL
S7	HPV39	400 copies/mL		S16	HPV66	400 copies/mL
S8	HPV45	400 copies/mL		S17	HPV73	400 copies/mL
S9	HPV51	400 copies/mL		S18	HPV82	400 copies/mL

Results: The test results of the 18 sensitivity reference products tested by the kit should be all positive and the coincidence rate should be 100%.
2.Analysis Accuracy
The samples of HPV16, HPV18, HPV26, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV68, HPV59, HPV66, HPV73 and HPV82 are prepared according to the preparation method in "National Reference Materials for L1 gene of Human Papillomavirus genotyping" as the samples to be tested and they are used as sensitivity samples P1-P18. The concentrations are shown in the following table:

No.	Type	Concentration		No.	Type	Concentration
P1	HPV16	1 x 10^3 copies/mL		P10	HPV52	1 x 10^3 copies/mL
P2	HPV18	1 x 10^3 copies/mL		P11	HPV53	1 x 10^3 copies/mL
P3	HPV26	1 x 10^3 copies/mL		P12	HPV56	1 x 10^3copies/mL
P4	HPV31	1 x 10^3 copies/mL		P13	HPV58	1 x 10^3 copies/mL
P5	HPV33	1 x 10^3 copies/mL		P14	HPV68	1 x 10^3 copies/mL
P6	HPV35	1 x 10^3 copies/mL		P15	HPV59	1 x 10^3 copies/mL
P7	HPV39	1 x 10^3 copies/mL		P16	HPV66	1 x 10^3 copies/mL
P8	HPV45	1 x 10^3 copies/mL		P17	HPV73	1 x 10^3 copies/mL
P9	HPV51	1 x 10^3 copies/mL		P18	HPV82	1 x 10^3 copies/mL

Results: The test results of the 18 accuracy reference products tested by the kit should be all positive and the coincidence rate should be 100%.
3.Analysis Specificity
Specific samples: For samples 1-25, refer to the 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 the species or cause similar symptoms, such as cytomegalovirus, herpes simplex virus II, Treponema pallidum, Ureaplasma urealyticum, Mycoplasma hominis, Neisseria gonorrhoeae, Chlamydia trachomatis, *Candida albicans*, and Trichomonas vaginalis in the "National Reference Materials for L1 gene of Human Papillomavirus genotyping". All 25 copies of samples should be confirmed to be positive for the corresponding pathogen by the corresponding kit.

No.	Type	Concentration		No.	Type	Concentration
1	HPV6	1 × 10^4 copies/mL		14	HPV81	1 × 10^4 copies/mL
2	HPV11	1 × 10^4 copies/mL		15	HPV83	1 × 10^4 copies/mL
3	HPV40	1 × 10^4 copies/mL		16	CP8304	1 × 10^4 copies/mL
4	HPV42	1 × 10^4 copies/mL		17	Cytomegalovirus	1 × 10^6 copies/mL
5	HPV43	1 × 10^4 copies/mL		18	Herpes simplex virus II	1 × 10^6 copies/mL
6	HPV44	1 × 10^4 copies/mL		19	Treponema pallidum	1 × 10^6 copies/mL
7	HPV54	1 × 10^4 copies/mL		20	Ureaplasma Urealyticum	1 × 10^6 copies/mL
8	HPV61	1 × 10^4 copies/mL		21	Mycoplasma hominis	1 × 10^6 copies/mL
9	HPV67	1 × 10^4 copies/mL		22	Neisseria gonorrhoeae	1 × 10^6 copies/mL
10	HPV69	1 × 10^4 copies/mL		23	Chlamydia trachomatis	1 × 10^6 copies/mL
11	HPV70	1 × 10^4 copies/mL		24	Candida albicans	1 × 10^6 copies/mL
12	HPV71	1 × 10^4 copies/mL		25	Trichomonas vaginalis	1 × 10^6 copies/mL
13	HPV72	1 × 10^4 copies/mL				

Result: The above-mentioned specific samples were all negative by this kit, which was in line with the intended use of the kit.
4. Precision
The HPV18 sample should be prepared with reference to the preparation method in the " National Reference Materials for L1 gene of Human Papillomavirus genotyping"" with the concentration of 1x10^3 copies/mL, as the precision reference material R, as shown in the following table:

No.	Type	Concentration
R	HPV18	1 × 10^3copies/mL

Results: The intra-assay precision variation coefficient CV should be less than or equal to 5%.

Precautions

1.The kit must be used within the validity period.
2. Do not open the iCassette cover before use. If you open it, you must use it within 30 minutes.
3. If you find that the iCassette is oscillated after adding the sample to cause leakage, please do not continue to use it.
4. The experimenter should prevent the sample from being contaminated. Take good protection and wear disposable gloves and masks.
5. Each iCassette is single-used, please do not reuse it.
6. In order to avoid any potential biological hazards in the samples, the test samples should be considered as infectious substances and avoid contact with skin and mucous membranes; sample manipulation and processing must comply with relevant regulations.

References

1.Agorastos T,Diinas K,Lloveras B,et al.Human papilloma virus testing for primary screening in women at low risk of developing cervical canceer The Green experience[J].Gynecol Oncol,2005,96(3):714-720.
2. Xi LF,et al.Viral load in th natural history of human papillomavirus type 16 infection:a nested case-control study.J Infect.Dis.2011,203:1425-1433
3. De Sanjose S,Quint WG,Alemany L et al.Human papillomavirus genotype attribution in invasive cervical cancer a retrospective cross-sectional worldwide study.Lancet Oncol.2010,11(11):1048-56

Instruction Version

Version: A/0
Date of Issue: October,2021

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		

IGENESIS(SHANGHAI)CO. , LTD.
Address: Floor 3, building 1, Lane 500, Furonghua Road, Pudong New Area, 201318 Shanghai, P.R. China.
Tel: +86-21-38016598

EC REP **Lotus NL B.V.**
Address: Koningin Julianaplein 10, 1e Verd,2595AA, The Hague, Netherlands.
Email：peter@lotusnl.com