ultrassay

Human Papillomavirus (HPV) Detection Kit (Real-time PCR)

Instruction for Use (V2.0.6)

[REF] UBP-C00448G/600170

[Specification] 48 tests/kit (Lyophilized)

[Intended Use]

This kit is intended for the in-vitro qualitative detection of nucleic acids of 26 HPV types (16、18、31、33、35、39、45、51、52、56、 58、59、68、26、53、66、73、82、6、11、40、42、43、44、54、81) in cervical exfoliated cells, and genotyping for 26 HPV types.

[Summary]

Human Papilloma virus (HPV), belonging to the Papillomaviridae family, is a small, circular, non-enveloped, double-stranded DNA virus, with a genome length of about 8,000 base pairs (bp). HPV infection is very common in women. About 80% of women have been infected with HPV in their lifetime, and 90% of them can be naturally cleared within one to two years. Based on their oncogenic potential, HPVs are classified as low-risk, associated with benign warts or epithelial lesions, or high-risk, that can cause oropharyngeal and anogenital malignancies, including cancers of the cervix, vulva, vagina, penis, and anus. High-risk HPV (hr-HPV) types are responsible for ~5% of all human cancers and are detected in 99.7% of cervical cancer cases, the fourth most common cancer in women, accounting for 7.5% of all cancer-associated deaths in women worldwide per year.

[Test Principles]

This kit uses specific primers and fluorescent probes for 26 types of human papillomavirus, using polymerase chain reaction (PCR) and Taqman technology to detect specific DNA fragments for 26 human papillomavirus types, respectively. The PCR amplification reaction system of this kit also contains the Uracil-N-Glycosylase and can selectively break the uracil glycosiside bond in the PCR fragment containing dUTP, effectively reducing false positives due to contamination of the PCR product.

[Kit Contents]

Table 1 Kit Contents

No.	Name	Specification	Description
1	HPV Reaction Strips	8-strips × 48	PCR buffer, primers, probes, dNTPs, Taq DNA Polymerase, Uracil-N-Glycosylase
3	Positive Control	100μL × 1	Plasmid DNA
4	Tube Caps	8-strips × 48	-

Noted: *The contents in different batches of reagents are not interchangeable within the shelf life.*

The order of the holes is shown in Figure 1

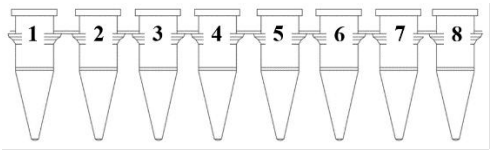

Figure 1 Hole layout diagram

Note: Starting from one end with a short black or blue line, tube 1, 2...8.

Materials required but not provided:

① Nuclease-free water.

② UltraPx Sample Extraction Solution or UltraPx Viral DNA/RNA Extraction Kit or other equivalent kits.

③ 1.5 mL RNase-and DNase-free centrifuge tubes, RNase-free and DNase-free tips, desktop centrifuge, Thermostatic water bath / dry bath and vortex mixer.

[Storage Conditions]

1. Store the kits and reagents at RT+2~35°C. HPV Reagent Strip (Freeze-dried) and Positive Control (Freeze-dried) can be transported at room temperature.
2. The dissolved positive control needs to be stored at -20±5℃ for not more than 1 year.
3. The shelf-life of the kit is 12 months. After disassembling, it is recommended to store tightly.
4. Always check the expiration date before use, and do not use any expired reagent. For the manufacturing date and expiration date, see the outer packaging box.
5. After opening, the Kit is stable up to the expiration date indicated on the packaging provided that the components have been stored correctly according to the recommendations

[Warnings]

1. Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
2. Please check the compatible real-time PCR instruments prior to use.
3. DO NOT use the kit or any kit component after their expiry date.
4. DO NOT use any other reagents from different lots in the tests.
5. DO NOT use any other reagents in the other test kits.
6. Collected samples should be tested as soon as possible and it can be stored at room temperature for 14 days without affecting the test results.

[Precautions]

1. Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
2. Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
3. Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
4. The HPV reagent strip is for in vitro diagnostic use only.
5. Use only one swab per strip to avoid contamination.
6. Do not use the HPV reagent strip beyond its expiration date.
7. Do not touch the reagent pad with fingers or any other object.
8. Do not use if the packaging is damaged or if the product appears to be defective.
9. We recommend centrifugation before use to concentrate all reagents at the bottom.

[Applicable Equipment]

Applied Biosystems 7500 Real-Time PCR Systems, Bio-Rad CFX96 Real-Time PCR System, UltraDx eQ9600 Real Time qPCR System.

[Acceptable Specimens]

Before sampling, gently wipe the excessive secretions of the cervix with a cotton swab, replace the cotton swab, use a cotton swab infiltrated with cell preservation solution or a sampling brush for cervical exfoliated cells to close to the cervical mucosa, and turn clockwise for 3-5 cycles to obtain cervical exfoliated cells. Slowly take out the cotton swab or brush, and put into the sample tube with 1 ml of Hanks or other preservation mediums. After rinsing thoroughly, squeeze the cotton swab or brush against the wall and discard. Tighten the cap, and mark the sample name (or number) and type on the sample tube.

2. Storage

The sample n be tested should not be stored at 2-8°C for more than 48 h, should be stored below-18°C for no More than six months, and can be stored below-7o'C for a long time.

[Test Procedures]

1. Reagent preparation

1) Take out the Positive Control (Freeze-Dried) from the kit. Transfer it to the sample processing area.

2) Take out the (n+2) HPV Reagent Strip (Freeze-Dried), tear open the packaging bag, and place the 8-tube strips labeled with numbers 1 to 8, aligning the numbers, on the PCR rack, and remove the silicone plug.

Note:

a. "n" is the sample size, "2" for NC and PC;

b. Each PCR test must contain one PC (positive control) and one NC (negative control).

3) Add 20μL of Nuclease-Free Water into tube 1 to 8 of HPV Reagent Strip (Freeze-Dried) and close the Tube cap to transfer the reagent to the sample handling area. Mark strips with S1, S2…… Sn, NC, PC. The layout order is as follows:

Table 2 Reagent preparation layout

	S1	S2	……	Sn	NC	PC
A	1	1	1	1	1	1
B	2	2	2	2	2	2
C	3	3	3	3	3	3
D	4	4	4	4	4	4
E	5	5	5	5	5	5
F	6	6	6	6	6	6
G	7	7	7	7	7	7
H	8	8	8	8	8	8

2. Sample preparation and Positive Control preparation

1) Please refer to the instructions when extracting nucleic acids.

1.1) If sample preservation medium that without lysis function, use UltraPx sample extraction solution (No.600211). Aspirate 1mL of sample, centrifuge at 12000rpm for 2 mins, discard the supernatant, and add 100uL sample extraction solution, 95°C metal bath for 10 mins, centrifuge at 12000rpm for 2 mins, and supernatant into a new tube.

1.2) If sample preservation medium that with lysis function was used, use UltraPx Viral DNA/RNA Extraction Kit or other suitable extraction kit and refer to their instructions.

2) Add 600uL nuclease-free water to Positive Control (Freeze-Dried), then vortexed for 2 mins. Dissolved Positive Control should be vortexed and mixed well before use.

Note: *The Dissolved Positive Control needs to be stored at -20±5 ℃.*

3) The DNA can be stored at 4°C for 2 days and needs to be tested as soon as possible. The DNA can be stored at -20 °C for 1 month.

3. Add samples

1) Take out (n+2) HPV Reagent strips ("n" for samples, "2" for NC and PC), and Centrifuge at 3,000 rpm for 1 min to concentrate all the reagent at the bottom. Mark strips with S1, S2……Sn, NC, PC. Then gently uncover the caps prior to use.

Prepare S1 strip for sample 1: Add 5 μL DNA of sample 1 into Tube 1-8, and cap the PCR tubes. Prepare other sample strip, NC and PC strip in the same manner as for sample 1. The layout order is as follows:

Table 3 Adding samples Layout

	S1	S2	……	Sn	NC	PC
A	Sample1	Sample2	……	Sample n	NC	PC
B	Sample1	Sample2	……	Sample n	NC	PC
C	Sample1	Sample2	……	Sample n	NC	PC
D	Sample1	Sample2	……	Sample n	NC	PC
E	Sample1	Sample2	……	Sample n	NC	PC
F	Sample1	Sample2	……	Sample n	NC	PC
G	Sample1	Sample2	……	Sample n	NC	PC
H	Sample1	Sample2	……	Sample n	NC	PC

2) Centrifuge at 3,000 rpm for 1 min to concentrate all the reagent at the bottom.

3) Place the HPV Reagent Strip into the real-time PCR system and set up the reaction program according to Table 4:

Table 4 Cycling Parameters

Step	**No. of Cycle**	**Temperature**	**Duration**	**Collect Fluorescent Signal**
1	1	37°C	5 mins	No
2	1	95°C	5 mins	No
3	45	95°C	15 secs	No
		60°C	30 secs	Yes

4) Instrument Setup

· Setup the reaction volume as 25ul.

· For ABI instruments please set up as follows: Reporter Dye: FAM, VIC, ROX, CY5; Quencher Dye: None; Passive Reference: NONE.

· For Bio-Rad CFX 96, If 8-Tube Strips (White) was used, please set up as follows: Reporter Dye: FAM, VIC, ROX, CY5, During the result analysis, open the "Quantification" window, Remove the "log scale", open "Baseline Threshold", set "Single Threshold"- User Defined:1000". If 8-Tube Strips (Transparent) was used, please set up as follows: Reporter Dye: FAM, VIC, ROX, CY5, During the result analysis, open the "Quantification" window, Remove the "log scale", open "Baseline Threshold", set "Single Threshold"-" User Defined:200".

· Refer to the real-time PCR instrument operator's manual for detailed instructions.

· We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

6) Start the PCR run immediately.

7) When the PCR run finished, analyze the data according to the "Results Interpretation" procedures.

[Explanation of Test Results]

1) The positive control and negative control should be included per PCR detection.
2) Negative control (FAM, VIC, ROX, CY5 channels have no Ct value) and Positive control (Ct values ≤39 in the FAM, VIC, ROX, and CY5 channels) must be satisfied in the same experiment, otherwise, the results of this experiment are invalid and need to be re-run.
3) If the positive control or bank control do not meet the criteria, the entire test is invalid, and results should not be reported. Operators should repeat the entire process (specimen and control preparation, amplification, and detection). If the repeated test is still invalid, please contact Technical Support.
4) Additional controls could be used in accordance with the requirements of local, state, federal accrediting organizations, if applicable.

Table 5 Results Interpretation

	FAM Channel		**VIC Channel**		**ROX Channel**		**CY5 Channel**	
	CT≤39	CT>39	CT≤39	CT>39	Ct≤39	CT>39	Ct≤39	CT>39
A	HPV11	-	HPV16	-	HPV6	-	β-globin	N/A
B	HPV18	-	HPV26	-	HPV56	-	HPV54	-
C	HPV31	-	HPV33	-	HPV35	-	β-globin	N/A
D	HPV39	-	HPV42	-	HPV43	-	HPV44	-
E	HPV45	-	HPV51	-	HPV58	-	β-globin	N/A
F	HPV52	-	HPV53	-	HPV81	-	β-globin	N/A
G	HPV59	-	HPV66	-	HPV73	-	β-globin	N/A
H	HPV68	-	HPV82	-	HPV40	-	β-globin	N/A

5) For the above A-H 8 kinds of human papillomavirus mixture FAM, VIC, ROX, CY5 channels,

6) The samples with channel measurement Ct>39, and β -globin (Ct≤39), report the corresponding human papillomavirus type negative.

7) The sample with channel measurement Ct≤39, and β -globin (Ct≤39), report the corresponding human papillomavirus type positive.

8) The samples with channel measurement Ct≤39, but β -globin (Ct >39 or no Ct value), resampled and do the experiment again.

9) The sample with channel measurement Ct > 39, and β-globin (Ct > 39 or no Ct value), the test result is invalid, find the reason, resampled and do the experiment again.

[Product Performance]

1. In the tests of positive reference controls, the positive coincidence rate was 100%.
2. In the tests of negative reference controls, the negative coincidence rate was 100%.
3. Limit of detection (LoD) of the kit is 1×10^3 copies/mL (50 copies/reaction)
4. Precision reference controls were tested 10 times, and the coefficient of variation (CV, %) of the Ct value was no greater than 5%.

[Limitations of Test Method]

1. A negative result cannot exclude the possibility of HPV infection and should not be used as the only basis for clinical assessment and treatment of patients.
2. Reliable results are dependent on the appropriate specimen collection, transport, storage, and processing procedures.
3. Inhibitors present in the specimen and/or errors in following the test procedure may lead to false negative results.
4. A trained health care professional should interpret test results with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
5. Potential mutations within the target regions of the virus genome covered by the tests primers and/or probes may result in failing to detect the presence of the pathogens.
6. False positive values may result from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the test.
7. The Human Papillomavirus (HPV) Genotyping Real Time PCR Kit is designed to augment existing methods for the detection of cervical disease and should be used in combination with clinical information derived from other diagnostic and screening tests, physical examinations, and full medical history in accordance with appropriate patient management procedures.
8. Infection with HPV is not an indicator of the cytologic high-grade squamous intraepithelial lesion (HSIL) or underlying high-grade cervical intraepithelial neoplasia (CIN), nor does it imply that a CIN 2-3 or cancer will develop. Most women infected with one or more high-risk HPV types do not develop CIN 2-3 or cancer.
9. A negative result cannot exclude the possibility of future cytologic HSIL or underlying CIN 2-3 or cancer. A small proportion of high-grade lesions occur in women who are HPV negative by existing technologies.

[References]

1. Gravitt PE, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol. 2000 Jan; 38(1):357-61.
2. Bosch FX, et al. Papillomavirus research update: highlights of the Barcelona HPV 2000 international papillomavirus conference. J Clin Pathol 2001; 54:163–175.
3. Weimin QU, et al. PCR Detection of Human Papillomavirus: Comparison between MY09/MY11 and GP5+/GP6+ Primer Systems. Journal of Clinical Microbiology, June 1997, p. 1304–1310.
4. François Coutlée, et al. Use of PGMY Primers in L1 Consensus PCR Improves Detection of Human Papillomavirus DNA in Genital Samples. Journal of Clinical Microbiology, Mar. 2002, p. 902–907.

[Index of Symbols]

Symbol	**Used for**	**Symbol**	**Used for**
	Use-by date		Consult instructions for use or consult electronic instructions for use
LOT	Batch code	IVD	In vitro diagnostic medical device
	Temperature limit		Manufacturer
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC	EC REP	Authorized representative in the European Community
REF	Catalog number	Σ	Contains sufficient for <n> tests
	Date of manufacture		Do not use if package is damaged and consult instructions for use

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