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

Instruction for Use (V1.1)

[REF] UBP-C00948G

[Specification] 48 tests/kit

[Intended Use]

This kit is intended for the in-vitro qualitative detection of nucleic acids of 18 high-risk HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) in cervical exfoliated cells, and genotyping for 16 and 18 HPV types. An additional primer pair and probe target the human β-globin gene to provide a process control.

[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 (hrHPV) 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]

It was used for qualitative detection of 18 high-risk human papillomavirus nucleic acid DNA (HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) in vitro in cervical exhumation cells, and typing of HPV16 and 18 types.

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]

No.	Name	Specification	Description
1	Reaction Well (with Lyophilized Reagent)	8 wells × 6	PCR buffer, primers, probes, dNTPs, Taq DNA Polymerase, Uracil-N-Glycosylase
2	Nuclease-free water	2mL x 1	Nuclease-free water
3	Positive Control	400μL × 1	Plasmid DNA
4	Negative Control	400μL × 1	Nuclease-free water

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

Reagents required but not provided:

① Viral DNA/RNA Extraction Kit produced by Ultrassay or other equivalent kits.

② 1.5 mL RNase-and DNase-free centrifuge tubes, RNase-free and DNase-free tips, desktop centrifuge, and desktop oscillation mixer.

[Storage Conditions]

1. Store the kits and reagents at room temperature.
2. The shelf-life of the kit is twelve months. After disassembling, it is recommended to store tightly.
3. Keep HPV reaction mix away from light.
4. Properly thaw and mix before reagent preparation.
5. 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.
6. 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.

[Applicable Equipment]

Applied Biosystems 7500 Real-Time PCR Systems, Ultrassay eQ9600 Real Time qPCR System or equivalent systems.

[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 sterile saline. 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℃ for no More than six months, and can be stored below-7o'C for a long time. Avoid repeated freezing and thawing.

3. Transportation

It is recommended to adopt ultra-low temperature transportation with liquid nitrogen and dry ice.

[Test Procedures]

1. DNA Extraction

1) If UltraPx Sample Extraction Solution used as the cell preservation solution, DNA extraction is unnecessary.
2) If not, recommended use UltraPx Nucleic Acid Extraction Kit. Referring to the Instruction for Use.

2. Detection

1) Take Reaction Well, Negative Control, Positive Control, Nuclease-free water, out of the kit from the Packaging bags.
   a) Every PCR run must contain one PC (Positive control) and one NTC (No template control).
   b) The prepared mixtures should be used immediately, avoid prolonged storage.
2) Dispensing 20 uL Nuclease-free water to (n+2) reaction strips.
3) Take out the sample DNA and nuclease-free water for NTC.
4) Add 5 uL NTC, 5 uL each sample DNA, 5 uL PC to a PCR tube with 20 uL HPV Master Mix respectively and cap the PCR tubes.
5) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
6) Place the PCR tubes into the appropriate positions of the real-time PCR instrument.
7) Setup the PCR Protocol using the cycling parameters in Table 2

Table 2 Cycling Parameters

No.	Stage	Temperature	During	No. of Cycle
1	UNG Treatment	37℃	5 Mins	1
2	Pre-denaturation	95℃	5 Mins	1
3	Denaturation	95℃	15 Secs	45
	Annealing, extension, and fluorescent signal collection*	60℃	30 Secs	

8) Start the PCR run immediately.
9) When the PCR run finished, analyze the data according to the "Results Interpretation" procedures.

[Explanation of Test Results]

The positive control and negative control should be included per PCR detection.

Negative control (FAM, VIC, ROX, CY5 channels have no Ct value) and Positive control (Ct values≤38 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.

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.

Additional controls could be used in accordance with the requirements of local, state, federal accrediting organizations, if applicable.

FAM:26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82.

VIC: 16.

ROX: 18.

CY5: β-globin.

1. The samples with channel measurement Ct >40, and β-globin (Ct ≤35), report the corresponding human papillomavirus type negative.

2. The sample with channel measurement Ct≤40, and β-globin (Ct ≤35), report the corresponding human papillomavirus type positive.

3. The samples with channel measurement Ct≤40, but β-globin (Ct >35 or no Ct value), resampled and do the experiment again.

4. The sample with channel measurement Ct > 40, and β-globin (Ct > 35 or no Ct value), the test result is invalid, find the reason, resampled and do the experiment again.

[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. Positive test results indicates the presence of any one or more of the high-risk types, but since patients may be co-infected with low-risk types it does not rule out the presence of low-risk types in patients with mixed infections.

3. Reliable results are dependent on the appropriate specimen collection, transport, storage, and processing procedures.

4. Inhibitors present in the specimen and/or errors in following the test procedure may lead to false negative results.

5. 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.

6. 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.

7. 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.

8. 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.

9. 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.

10. 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.

[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 2×103 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%.

[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.

[References]

1. Gravitt PE, etal. 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]

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		Any warnings and/or precaution to take

Ultrassay Biotech Co., Ltd.

Add: 2906, Building B, Dongyi Square, No. 169 Funan Road, Luyang District, Hefei, Anhui, China 230061

Tel: +86-551-6288 1663

Website: Http://ultrassay.com

EC REP Company: SUNGO Cert GmbH

Address: Harffstr. 47,40591 Düsseldorf, Germany

Tel /Fax: +49(0)211 97634133

E-mail: de.rep@sungogroup.com

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