208 Denise Cir Newmarket, ON L3X 2K2
CANADA Tel: 416-409-5207
info@canadasmarttech.com
Canada Smart Tech

HUMAN PAPILLOMAVIRUS 24 GENOTYPES LYOPHILIZED DETECTION KIT

(Fluorescent Probe-based Real-time PCR Assay)

Instruction for Use

Smart HPV 24 Lyo

CE

IVD

Number: S8725

Effective Date: XXXXXXXX

PRODUCT NAME

Human Papillomavirus 24 Genotypes Detection Kit (Fluorescent Probe-based Real-time PCR Assay)

INTENDED USE

This kit is intended to qualitatively detect the deoxyribonucleic acid (DNA) of 24 types of human papillomavirus (HPV) in women's cervical shedding cell samples, and identify HPV as 16, 18 ,31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 26, 53, 66, 73, 82, 6, 11, 42, 43, 44 or 81 genotypes. It is used for assisted diagnosis of HPV infection. For professional in-vitro diagnostic use.

CONTRAINDICATIONS

The efficacy of this test has not been assessed for the guidance of women who have undergone ablative or excisional therapy, hysterectomy, are currently pregnant, or possess additional risk factors such as being HIV positve, or having a history of STI.

SUMMARY AND EXPLANATION

Human papillomaviruses are small, non-enveloped, spherical DNA viruses with a diameter of 52–55 nm. The viral particles consist of a single double-stranded circular DNA which is 8000 base-pairs (bp) and with a shape of regular icosahedron. Papillomaviruses are highly epitheliotropic, with a highly host-specific affinity and human beings are the only host of HPV.

The types of 6, 11, 42, 43, 44 and 81 can cause the skin mucosa wart-like lesions. According to the research results of the WHO International Agency for Research on Cancer (IARC), HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 genotypes are classified as high-risk types, and HPV 26, 53, 66, 73, 82 genotypes are classified as middle-risk types.

TEST PRINCIPLE

The primers and fluorescent probes of this kit are specifically designed in the E6/E7 loci of HPV genome, and the fluorescent probes are labeled with FAM, HEX/VIC, and ROX fluorescent dyes, respectively. By using Polymerase Chain Reaction (PCR) with TaqMan fluorescent probes, qualitatively detect the 24 types of HPV DNA from the samples of women's cervical shedding cells. It also contains endogenous Internal Control ,and the probe is labeled with Cy5 fluorescent dye.

REAGENTS PROVIDED

Seq.	Label	Main Contents	No. (24 tests)
1	PCR reagents strips	Well 1 of the reagents strip: Mixture of dNTPs, magnesium, HPV 16, 18, 31 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	24 strips
		Well 2 of the reagents strip: Mixture of dNTPs, magnesium, HPV 33, 35, 39 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	
		Well 3 of the reagents strip: Mixture of dNTPs, magnesium, HPV 45, 51, 52 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	
		Well 4 of the reagents strip: Mixture of dNTPs, magnesium, HPV 56, 58, 59 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	
		Well 5 of the reagents strip: Mixture of dNTPs, magnesium, HPV 68, 26, 53 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	
		Well 6 of the reagents strip: Mixture of dNTPs,	

		magnesium, HPV 66, 73, 82 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	
		Well 7 of the reagents strip: Mixture of dNTPs, magnesium, HPV 6, 11, 42 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	
		Well 8 of the reagents strip: Mixture of dNTPs, magnesium, HPV 43, 44, 81 and Internal Control primers and probes, UDG and Taq DNA polymerase, etc.	
2	Positive Control	Fragments of detected 24 types of HPV E6/E7 gene and Internal Control gene	1 tube (1.3mL/tube)
3	Negative Control	Fragment of Internal Control gene	1 tube (1.3mL/tube)
4	Nuclease-free Water	Nuclease-free Water	2 tube (1.7mL/tube)
5	8 Strip Caps	/	24 pieces

OTHER MATERIALS REQUIRED BUT NOT PROVIDED

The following list includes the materials that are required for use but not included in this Kit:

- Nucleic acid extraction kit.
- Nuclease-free consumables: Filter tips, 1.5mL tubes.
- Experimental equipment: Centrifuge for 1.5mL tubes and PCR-well strips or 96-well plate (if available), Vortex. Real Time PCR instrument (thermocycler).
- Others: Micropipettes (0.5-20μL, 10-100μL, 20-200μL,100-1000μL), Powder-free disposable gloves.

ACCEPTABLE SPECIMENS

The samples for testing are women's cervical shedding cells.

SPECIMENS COLLECTION AND STORAGE

The samples collected by using cervical shedding cell collectors. (*e.g.,* disposable use of sterile cervical sample collector, pay attention to sterile operation in the process of sampling. Not provided in this kit.). The operation steps are as follows:

1. The medical staff exposes the cervix with a vaginal dilator and wipes off the secretions with a disposable swab.
2. Insert the cervical brush into the cervix and gently rotate it for 4-5 times.
3. Pull out the cervical brush slowly and put it into the tube containing solution.Avoid repeated freezing and thawing of the clinical specimens during the transport and storage process. The samples should be transported at least below 8 °C, if the transportation cannot be guaranteed at - (20±5) °C. The shelf life of the samples at 2-8 °C is 7 days, - (20±5) °C is 12 months. The accurate clinical information of the samples, such as specimen number, date of onset and date of collection, should be attached with the transport and preservation process.

STORAGE CONDITIONS AND SHELF LIFE

The shelf life of this kit is 24 months from the manufacturing date when stored at 2-8 °C. It is suggested to transport the kits below 42 °C. Never leave the kit for more than 30 days at 42 °C. Please keep the kit under light avoidance.

Upon opening the sealed pouch, please consume the test promptly. If unable to consume them all at once, store the remaining test at a temperature of -20±5°C for a maximum of 15 days.

APPLICABLE EQUIPMENT

Applicable to ABI 7500 Real-Time PCR thermocycles, Bio-Rad CFX 96, and for other Real-Time PCR thermocyclers, please consult the manufacturer/your distributor before use.

TEST METHODOLOGY

1. Nucleic Acid Extraction (Pre-PCR)

Extract the nucleic acids from the clinical samples, positive control and negative control according to the instructions of the nucleic acid extraction kit:

- Clinical sample: Prepare the samples according to the instructions for use of extraction kit.
- Positive Control: Prepare the samples according to the instructions for use of extraction kit.
- Negative Control: Prepare the samples according to the instructions for use of extraction kit.

* Please ensure to verify the compatibility of nucleic acid kits from other suppliers before use.

Preparation of Amplification Reagent (PCR Room I)

Take out the reagents from the kit and remove the package, centrifuge at 3000-6000rpm for 10s to make the pellet concentrate at the bottom of the well. **Align the strips in a consistent direction, starting from well 1 and progressing through well 8, with each well of the PCR strips marked with numbers 1-8**, put the PCR reagents strips (HPV 24) on a plate-matched shelf carefully (Note: when taking a strip, always wear PE gloves or powder-free latex gloves to avoid polluting the exterior of tube which may affect the test.). Remove the caps carefully, and then **add 15μL Nuclease-free Water to each well**, then transfer the tubes to the PCR room II.

Add the Templates (PCR Room II)

Add **5.0μL** extracted nucleic acid samples and quality controls (prepared in the first step: Pre-PCR) into each well containing the PCR reagents, cover with new 8 strip caps. Vortex the sealed the strips to mix well and then centrifuge at 2000-3000rpm for 20s.

Example: Extract 10 nucleic acids from 10 clinical samples, as well as positive negative control, and label them as S1~S10, PC and NC,

respectively. Take 12 PCR reagents strips (HPV 24), align the strips in a consistent direction, starting from well 1 and progressing through well 8, add 15.0μL Nuclease-free Water to each well, then add 5.0μL nucleic acids into each well in each column respectively in PCR room II, e.g., add S1 to each well in A1~H1, add S2 to each well in A2~H2, etc. Centrifuge after sealing the strips.

Reagents strip	location	1	2	3	4	5	6	7	8	9	10	11	12
Well 1	A	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
Well 2	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
Well 3	C	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
Well 4	D	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
Well 5	E	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
Well 6	F	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
Well 7	G	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC
Well 8	H	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	NC	PC

2. Amplification (Detection Area)

Place the strips in a unified direction from well 1 to well 8 into the fluorescent real-time PCR thermocycler and set the cycle program as follows:

Step	Cycles	Temperature (°C)	Time (min:sec)
1	1	50	05:00
2	1	95	05:00
3	5	94	00:10
		58	00:30
4	40	94	00:10
		56*	00:30

Fluorescent dye signals assigning: FAM, HEX/VIC, ROX, Cy5. *The signal data is collected at 56°C. When using the ABI7500, select the 'Quencher' and 'Passive reference' columns as "none". Set the

reaction volume per well to 20μL.

CUT-OFF VALUE

The cut-off value of FAM, HEX/VIC and ROX is 34.00 respectively and is determined by ROC curve. The cut-off value of internal control is determined to be 30.00 by limited dilution.

EXPLANATION OF THE TEST RESULTS

After the reaction is completed, the instrument automatically saves the results, adjusts the Start, End, and Threshold values of the Baseline after analyzing the image (It is user self-adjustable: Start values can be between 3 and 15. End values can be between 5 and 20).

- For negative control, the Ct value in Cy5 channel control should be ≤30.00, and Ct value in the other 3 channels should be negative.
- For positive control, Ct value in the 4 detected channels should be positive.

The above requirements must be met in the same test at the same time, otherwise the PCR reaction is considered invalid and should be re-performed.

When all the above requirements are met, the interpretation and judgment of test results are as following:

- When there is no amplification in all 4 detection channels of 8 mixtures, re-sampling or re-extraction is needed.
- When the Ct value in Cy5 channel is ≤30.00 with a typical S-type amplification curve, the interpretation of the results is listed in the table below:

Mixture	Dye/Ct	Type	Mixture	Dye/Ct	Type
Mix 1	FAM≤34.00	HPV 16 positive	Mix 5	FAM≤34.00	HPV 68 positive

	FAM＞34.00	HPV 16 negative		FAM＞34.00	HPV 68 negative
	HEX/VIC≤34.00	HPV 18 positive		HEX/VIC≤34.00	HPV 26 positive
	HEX/VIC＞34.00	HPV 18 negative		HEX/VIC＞34.00	HPV 26 negative
	ROX≤34.00	HPV 31 positive		ROX≤34.00	HPV 53 positive
	ROX＞34.00	HPV 31 negative		ROX＞34.00	HPV 53 negative
Mix 2	FAM≤34.00	HPV 33 positive	Mix 6	FAM≤34.00	HPV 66 positive
	FAM＞34.00	HPV 33 negative		FAM＞34.00	HPV 66 negative
	HEX/VIC≤34.00	HPV 35 positive		HEX/VIC≤34.00	HPV 73 positive
	HEX/VIC＞34.00	HPV 35 negative		HEX/VIC＞34.00	HPV 73 negative
	ROX≤34.00	HPV39 positive		ROX≤34.00	HPV 82 positive
	ROX＞34.00	HPV 39 negative		ROX＞34.00	HPV 82 negative
Mix 3	FAM≤34.00	HPV 45 positive	Mix 7	FAM≤34.00	HPV 6 positive
	FAM＞34.00	HPV 45 negative		FAM＞34.00	HPV 6 negative
	HEX/VIC≤34.00	HPV 51 positive		HEX/VIC≤34.00	HPV 11 positive
	HEX/VIC＞34.00	HPV 51 negative		HEX/VIC＞34.00	HPV 11 negative
	ROX≤34.00	HPV 52 positive		ROX≤34.00	HPV 42 positive

	ROX＞34.00	HPV 52 negative		ROX＞34.00	HPV 42 negative
Mix 4	FAM≤34.00	HPV 56 positive	Mix 8	FAM≤34.00	HPV 43 positive
	FAM＞34.00	HPV 56 negative		FAM＞34.00	HPV 43 negative
	HEX/VIC≤34.00	HPV 58 positive		HEX/VIC≤34.00	HPV 44 positive
	HEX/VIC＞34.00	HPV 58 negative		HEX/VIC＞34.00	HPV 44 negative
	ROX≤34.00	HPV 59 positive		ROX≤34.00	HPV 81 positive
	ROX＞34.00	HPV 59 negative		ROX＞34.00	HPV 81 negative

- In general, the internal control should show a typical S-type amplification curve. When the concentration of the target viral gene is too high, may cause a failure of the amplification of internal control. Therefore, any of other channel has positive result can be directly reported as a positive sample or dilute the sample and re-testing.

LIMITATIONS OF THE TEST METHOD

The test results of this kit are for clinical reference only and shall not be used as the sole basis for diagnosis or exclusion, it should be analyzed by combination with clinical symptoms/signs, medical history, treatment responses and results of other laboratory examinations. A negative result indicates the pathogen concentration in the sample is lower than the detection limitation of the kit, in this situation, the infection cannot be excluded.

The most appropriate specimen types and the highest virus titer time after infection have not been verified, therefore, several times,

multi-sites of sample collection in the same patient may help to avoid false negative results.

The following conditions can also cause a false positive or false negative test result:

1. The results can be affected by collecting, transporting and storage of samples, and any errors of these processes will result in false negative.
2. The mutation of sequence related to primers or probes used in this kit may cause false negative results.
3. Cross-contamination during sample processing may lead to false positive, as evidenced by the presence of amplification curves in the FAM or HEX/VIC or ROX channel of the negative control.

PRODUCT PERFORMANCE

1. The limit of detection (LoD): The limit of detection for all targeted HPV types are 500 copies•mL^{-1}.
2. Precision: for Intra batch, the coefficient of variation of Ct values of precision references is not higher than 5% and for inter-batch the coefficient of variation of Ct values is not higher than 10%.
3. Specificity
   - 3.1 The kit identified 24 types of HPV. No cross-reactivity was found among the detected 24 types of HPV.
   - 3.2 The kit did not show any cross-reactivity with any other microorganisms parasitized in human urinary and reproductive tract or sexually transmitted pathogens, including Herpes simplex virus type II, *Treponema pallidum*, *Ureaplasma urealyticum*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae* (*Gonorrhea*), *Candida albicans*, Trichomonas vaginalis, *Chlamydia trachomatis*. No cross-reactivity was found with other non-targeted HPV types such as 40, 54, 61, 67, 69, 70, 71, 72, and 83. (The tested

concentration of the above virus was 10^5 PFU•mL^{-1} or higher, the bacteria was 10^6 CFU/mL or higher, the mycoplasma was 10^6 CCU•mL^{-1} or higher, respectively).

3.3 Interfering substances: 10% leukocyte, 5% lubricant, 2% antibacterial lotion, 2mg•mL^{-1} hemoglobin, 0.1mg•mL^{-1} of mucin, 10mg•mL^{-1} Nonoxynol and 4mg•mL^{-1} Miconazole nitrate contained in the sample had no significant influence on the performance of the kit. However, still try to avoid any interference substances in the process of sampling.

PRECAUTIONS

1. Experimental personnel who perform this test should have had professional training in gene amplification or molecular biology diagnostics and be qualified for relevant experimental operations. There should be having reasonable biosecurity precautions and protective procedures in the laboratories. The test should only be performed in laboratories that follow safety practices according to the applicable Biosafety Regulations in Microbiological and Biomedical Laboratories.
2. The whole detection process should be carried out in three areas: the first area is for reagent preparation. The second area is for specimen processing and reaction system preparation. The third area is for amplification, fluorescence detection, and results analysis. Instruments, equipment, and lab coats should be used independently in each area to prevent contamination.
3. In the testing process, should always take care to avoid RNase contamination, wear disposable gloves without fluorescent substances (Frequent replacement is recommended), use the disposable thin-walled 200µL PCR tube (or 96-well PCR plate with optical film) and pipette tips with filter. Never touch the reaction tube directly with bare hands.

4. The handling of Clinical Specimens should be performed in the biosafety cabinet to ensure the safety of laboratory staff and prevent environmental pollution. Harmful and/or toxic specimens and reagents in the experiment should be properly placed and stored, and in charge by an assigned person. Waste should be disposed of properly in special containers. Lab bench, equipment such as operator's stations, pipettes, centrifuges, PCR thermocyclers, etc., should be regularly wiped and disinfected with 1.0% sodium hypochlorite and/or 70% ethanol. Laboratory room, an ultra-clean bench should be treated with an ultraviolet lamp regularly and after each experiment.
5. Before the experiment, PCR reagents strips should be centrifuged for a few seconds to bring down powder to the bottom of the 8-strip tube before use. When preparing the reaction solution, attention should be paid to mixing all liquids on the vortex mixer, not blowing with the pipette to avoid bubbles, and centrifuging the reaction mixture solution for a few seconds. Use the kit before the expiration date and do not mix reagents with different batch numbers.

REFERENCES

[1] Tommasino, M. The human papillomavirus family and its role in carcinogenesis[J]. Semin. Cancer Biol. 2014, 26,13–21.
[2] Tommasino, M. The biology of beta human papillomaviruses[J]. Virus Res. 2017, 231, 128–138.
[3] Letian T, Tianyu Z. Cellular receptor binding and entry of human papillomavirus[J]. Virology Journal. 2010, 7(1): 2-2.
[4] McBride, A.A. Oncogenic human papillomaviruses[J]. Philos. Trans. R. Soc. Lond. B Biol. Sci.
[5] Payan C, Ducancelle A, Aboubaker M, et al. Human Papillomavirus Quantification in Urine and Cervical Samples by

Using the Mx4000 and LightCycler General Real-Time PCR Systems[J]. Journal of Clinical Microbiology. 2007, 45(3): 897-901.
[6] Coutlee F, Rouleau D, Petignat P, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the linear array HPV genotyping test[J]. Journal of Clinical Microbiology. 2006, 44(6): 1998-2006.
[7] Harden, M.E.; Munger, K. Human papillomavirus molecular biology[J]. Mutat. Res. Rev. Mutat. Res. 2017, 772,3–12.
[8] Tabrizi S N. Quality assessment for human papillomavirus testing[J]. Sexual Health. 2010, 7(3): 335-337.
[9] Van Doorslaer, K.; Li, Z.; Xirasagar, S.; Maes, P.; Kaminsky, D.; Liou, D.; Sun, Q.; Kaur, R.; Huyen, Y.; McBride, A.A. The Papillomavirus Episteme: A major update to the papillomavirus sequence database[J]. Nucleic Acids Res. 2017, 45, D499–D506.

Manufacturing date and expiration date: view on label

INDEX OF SYMBOLS

	Consult Instructions for Use		Contain <n> tests		Temperature limit 2 to 8° C
IVD	In vitro diagnostic medical device		Use-by date	CE	CE conformity marking
REF	Catalogue #	LOT	Lot Number		
	Manufacture Date		Manufacturer		