Human Papillomavirus DNA Diagnostic Kit (Two Tube)

[Product Name]

Human Papillomavirus DNA Detection Kit (PCR-fluorescent Probe)

[Intended Use]

This kit is an ***in vitro*** diagnostic test used for the detection of the Human Papillomavirus (HPV). Samples can be obtained from cervix swab.

[Packaging Specification]

24 Tests/Pack; 48 Tests/Pack; 96 Tests/Pack

[Kit Contents and Components]

Table 1. Kit Contents

Catalog No.	DDP3108-24	DDP3108-48	DDP3108-96
Kit Size	24 Tests/Pack	48 Tests/Pack	96 Tests/Pack
A-Master Mix (HPV)	3 strips	6 strips	12 strips
B-Master Mix (HPV)	3 strips	6 strips	12 strips
Solvent(HPV)	550μL（2tubes）	1100μL（2tubes）	1100μL（4tubes）
A-Positive Control(HPV)	40μL	40μL	40μL
B-Positive Control(HPV)	40μL	40μL	40μL
Nuclease-free Water	0.5mL	0.5mL	0.5mL
PCR Activator	24μL	48μL	96μL

Table 2. Kit Components

Components	Main Compositions
A/B Master Mix (HPV)	Tris-HCL; Primers; Probes; Enzyme mix including UDG; Taq polymerase; dNTPs; RNasin
Solvent(HPV)	Tris-HCL; Glycerin; KCL; MgCl2
A/B Positive Control(HPV)	Synthetic DNA Fragments; Nuclease-free Water
Nuclease-free Water	Nuclease-free Water

Table 3. Labeled Probes for Specific Genes

Target (A-Master Mix)	Fluorescent Labels	Quencher Dye
HPV16	FAM	MGB
HPV18	VIC/HEX	BHQ2
HPV52	ROX	MGB
HPV (other High risk) 31/33/35/39/51/53/56/58/59/66/68/73/82	CY5	MGB

Table 4. Labeled Probes for Specific Genes

Target (B-Master Mix)	Fluorescent Labels	Quencher Dye
HPV (other Low risk) 40/42/43/44/54/61/74/81	FAM	MGB
HPV6 &HPV11	VIC/HEX	MGB
HPV45	ROX	BHQ2
Internal Control gene(RNase P)	CY5	BHQ1

Note:

1) Kit components of Master Mix is in lyophilized form and need to be reconstituted before use.

2) Positive Control consists of the mixture of synthetic single-stranded DNA, which needs to be dispensed to 5μL per reaction. Please avoid repeated freezing-thawing.

3) Kit components from different batch number are not to be used interchangeably.

4）Master Mix is the basic components of the kit, which contains specific primers and probes for HPV.

[Storage]

1. All reagents can be stored at room temperature during the specified shelf-life. The shelf-life can be extended by storing the kit at 2-8℃ or -20℃.
2. After reconstitution, the Master Mix can be kept at -20±5℃ for a month.
3. Protect Master Mix (Lyophilized) from light during storage.
4. Avoid repeated freezing-thawing for more than 5 times after reconstitution.
5. Shelf-Life: 12 months.

[Manufacturing Date and Expiration Date]

See details on packaging label.

[Materials and Devices Required but Not Provided]

1. This product can be used with the following appropriate real time PCR instrument: Ardent GS-PCR3200; ABI7500, ABI Quant Studio models 3/5/6/7/12K; Roche Lightcycler®480/1536/Nano; Agilent Mx3000P/3005P; Qiagen Rotor-Gene 6000/Q; Bio-Rad CFX384/CFX96, Bio-Rad Touch/iQ5; Cepheid Smart-cycler/Smart Cycler II; Eppendorf Master Cycler.
2. Always use RNase/DNase free water and RNase/DNase free PCR reaction tube/PCR reaction plate for the test.
3. PCR tubes or 96-well PCR plates.

[Procedure]

1. Prepare the sample DNA with DNA isolation kit according to the manufacturer instruction.
2. Formulation of PCR One-step Mix.

2.1 To reconstitute all lyophilized powder, gently spin the strip of the lyophilized Master Mix for a few seconds to move all powder to the bottom before adding reconstitution buffer.

2.2 Add 21μL of reconstitution buffer (Solvent) to each lyophilized Master Mix strip well.

2.3 Add 1μL of PCR Activator to the corresponding reaction well containing Master Mix.

2.4 Add 5μL of DNA sample/Positive control/Negative control to the corresponding reaction well containing Master Mix.

Table 5. Setup of Assay Kit Components

Reagents	Individual test (μL)
Master Mix (Dissolved)	21
DNA Sample	5
Total Volume	26

2.4 Seal the tube with cap and shake with inching on the shaker several times, followed by short spin on the centrifuge.

Note: After reconstitute Master Mix was added with the sample, the reagent should be put into PCR instrument for detection within 1 hour.

3. PCR protocol as below:

Table6. Setup of PCR Thermal Cycling

Temp (℃)	Time	Cycles
95	60sec	1
95	10 sec	45
58	30sec（Fluorescence collection）	

[Result Interpretation]

1. Quality Check for the Test Results:

The readings of Ct value of positive control and negative control within the same reaction plate need to be:

Table 7. Quality Control Check

	Quality control requirement
Positive Control	Ct≤40
Negative Control	Ct>40 or No Ct

2. The experiment is invalidated, if the positive control and/or negative control does not meet the criteria set above.

3. The analysis of the Ct value of the wells in each swab as follows:

Table 8. Result Interpretation

	A-Master MIX				B-Master MIX				
	FAM-HPV16	VIC/HEX-HPV18	ROX-HPV52	CY5-other High risk	FAM-other Low risk	VIC/HEX-HPV6 &HPV11	ROX-HPV45	CY5-β-Actin	Interpretasi Hasil
1	Ct≤40	-	-	-	-	-	-	Ct≤40	Infected HPV16
2	-	Ct≤40	-	-	-	-	-	Ct≤40	Infected HPV18
3	-	-	Ct≤40	-	-	-	-	Ct≤40	Infected HPV52
4	-	-	-	Ct≤40	-	-	-	Ct≤40	Infected HPV other High risk
5	-	-	-	-	Ct≤40	-	-	Ct≤40	Infected other Low risk
6	-	-	-	-	-	Ct≤40	-	Ct≤40	Infected HPV6&HPV11
7	-	-	-	-	-	-	Ct≤40	Ct≤40	Infected HPV45
8	-	-	-	-	-	-	-	Ct>40 or No Ct	Resampling for test
Example of determining multiple infection results									
9	Ct≤40	Ct≤40	-	-	-	-	-	Ct≤40	Infected HPV16 HPV18
10	Ct≤40	-	-	Ct≤40	-	-	-	Ct≤40	Infected HPV16 、other High risk
11	-	Ct≤40	-	Ct≤40	Ct≤40	-	-	Ct≤40	Infected HPV18、other High risk、HPVother Low risk

Note: (1)"-"No requirement ; Ct>40 or No Ct, Not detected.

（2）Other multiple infection determination results，Please refer to Case 9, 10, and 11.

（3）If the Ct value of the corresponding fluorescence channel is ≤40, it indicates a positive HPVtype for that fluorescence channel.

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[Kit feature and specification]

1. Limitation of Detection (LOD): 200 copies/mL.
2. Cross-reactivity: No cross reaction with 24viruses (HSV-2,Treponema Pallidum,MH,-candida Albicans,Trichomonas vaginalis,Chlamydia trachomatis,Gardnerella vaginal-is,Corynebacteriuum parvm,Acinetobacter baumannii,Mycolicibacterium smegma-tis,Bacterooides fragilis,Enterobacter cloacae,Enterococcus faecalis,Escherichia coli,S. aureus,Staphylococcus epidermidis,α -hemolytic streptococcus,Hepatitis B virus,Hepa-titis C virus,HIV,Epstein -Barr virus,Cytomegalovirus,herpes simplex virus) and human genome DNA
3. Internal precision: repeatability: CV< 10%, between-run precision: CV < 10%, between-day precision: CV< 10%, total precision: CV< 10%.
4. External precision: repeatability: CV< 10%, between-run precision: CV < 10%, total precision: CV < 10%.
5. Interference reaction: Five potential reference (Dexamethasone, Azithromycin, Tobramycln, Levofloxacin, Ceftriaxone) will not interfere with the detection results of the kit.

[Limitation]

1. Negative results cannot completely rule out the existence of HPV. Improper sample collection, improper transportation, improper processing and insufficient initiation VL (viral load) may influences the experimental results.
2. Other unverified interferences or PCR inhibitors may cause false negative results.

[Warnings and Precautions]

1. For ***in vitro*** diagnostic use only.
2. Carefully read this instruction before use. Components from different batch number cannot be used interchangeably.
3. After being reconstituted, the lyophilized components can be either used up or stored at -20±5℃ and can be kept for one month. Avoid repeated freeze- thaw for more than five times.
4. Viral DNA and PCR Master Mix are sensitive to temperature, and should always put on ice during experiment.
5. Always wearing gloves and mask during experiment to avoid microbial and nuclease (DNase/RNase) contamination of the specimen and the reagents of the kit.
6. Always use DNase/RNase-free disposable aerosol-blocking pipette tips.
7. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
8. Discard sample and assay waste according to your local safety regulations.
9. Do not use components of the kit after expiration date.

[Background Information]

Human papillomavirus (HPV) can infect the reproductive tract through a variety of ways, resulting in condyloma acuminatum and cervical lesions, and may even cause cervical cancer. It takes about 5 to 10 years to develop from persistent infection with high-risk HPV to common precervical lesions and ultimately cervical cancer. Therefore, HPV detection is of great significance for the early diagnosis and treatment of cervical cancer. This kit uses fluorescence PCR technology to design specific primers and probes based on the gene characteristics of 27 kinds of HPV. Target fragments of 27 HPV genotypes can be amplified by detecting nucleic acids in cervical swab samples.

The HPV subtypes detected by this kit include:

6,11,16,18,31,33,35,39,40,42,43,44,45,51,52,53,54,56,58,59, 61,66,68,73,74,81,82.

[Detection Principle]

In PCR-Fluorescent Probe method, the probe with specific binding to target sequence is added based on the forward primer and the reverse primer, Specific primers and probes are designed based on specific gene areas of Human papillomavirus (HPV). Probes consist of a reporter fluorophore at 5′and quenching fluorophore at 3′.The fluorescent signals emitted from reporter fluorophores are absorbed by the quenchers, so it doesn't emit signals. During amplification, probes bonded to templates are cut off by Taq enzyme (5′- 3′exonuclease activity), separating reporter dye from the quencher, generating fluorescent signals, the PCR instrument will then automatically draw a real-time amplification curve based on the signal change, finally realizing the qualitative detection ofHuman papillomavirus (HPV) at the nucleic acid level.

[Recommendation on Specimen according to the WHO Guideline] 1.

Applicable sample type:cervix swab .

2. Requirements on sample collection: the sample collection shall be conducted with polyester swab or polyester flocked swab.
3. Sample transportation and storage: the transportation and shipping conditions should be carried out according to the instruction of sample collection kits used. Long term storage should be in -20 ℃ or -70℃.

[Explanation of Marks]

Diagram and symbol used on kit label	Remarks
	Manufacturer
	Authorized representative in the European Community

Diagram and symbol used on kit label	Remarks
	Consult instructions for use
	*In vitro* diagnosis reagent
	Contains sufficient for <n> tests
	Date of manufacture
	Use-by date
	Do Not Reuse
	Batch code
	Biological risks
	Storage temperature
	Keep dry
	Keep away from sunlight
	Fragile, handle with care
	Recoverable PAP material
	Recoverable PP material
	Recycled recyclable
	CE mark

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