AmoyDx
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AmoyDx® Human Papillomavirus (HPV) Genotyping Detection Kit

Instructions for Use

For Research Use Only

REF 8.01.0093 48 tests/kit For Stratagene Mx3000P™, ABI7500, LightCycler480, SLAN-96S, Rotor-Gene Q (72 wells)

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Background

Human papillomavirus (HPV) is a sexually transmitted DNA virus that establishes infection in squamous epithelial cells in the human body. There are more than 200 types of HPV, which can be classified into high or low-risk types depending upon their oncogenic potentials. High-risk HPVs also called oncogenic HPVs, which have been confirmed to cause cancer, includes HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. And some HPVs are possibly carcinogenic to humans, like HPV26, 53, 66, 70, 73 and 82, that be classified as high-risk or probably high-risk HPVs. Low-risk HPVs can cause genital warts and low-grade changes in the cells, but rarely cause cancer, such as HPV6 and 11. High-risk HPV infection is a necessary for the development of cancers of the uterine cervix, which has been firmly established. Approximately 99.7% of cervical cancers are caused by high-risk HPV infection. Cervical cancer is the second-most frequent malignancy among women worldwide.

Intended Use

The AmoyDx® Human Papillomavirus (HPV) Genotyping Detection Kit a real-time PCR assay for qualitative detection of 19 high-risk HPV DNA (HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82) and two low-risk HPV DNA (HPV 6 and 11) in cervical exfoliated cells and urogenital tract secretion. The kit is intended to access the high risk HPV DNA and aid in cervical cancer screening, early diagnostics and treatment.

The kit is for research use only, and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit is designed for a specific amplification of L1 gene in HPV DNA. The targeted region of HPV DNA is amplified by several specific primers and detected by fluorescence probes. A non-rivalry internal control is added in the HPV DNA detection system to ensure proper PCR procedure.

The kit is composed of 8 **HPV^{19} Reaction Mixes, HPV^{19} Enzyme Mix** and **HPV^{19} Positive Control.**

1) The **HPV^{19} Reaction Mix 1~7** includes a HPV DNA detection system. It contains primers and fluorescent probes specific for HPV DNA.
2) The **HPV^{19} Reaction Mix 8** includes a HPV detection and internal control system. It contains primers and FAM-labeled probes specific for HPV6 DNA, CY5-labeled probe specific for HPV11 DNA and HEX-labeled probe specific for internal control. The internal control system is designed to detect a housekeeping gene as reference gene to assess the presence of inhibitors and confirm the validity of each experiment.
3) The **HPV^{19} Enzyme Mix** contains the Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which works at room temperature to prevent PCR amplicon carryover contamination.
4) The **HPV^{19} Positive Control (PC)** contains recombinant gene with HPV plasmid DNA.

Kit contents

The kit contains the following materials (Table 1).

Table 1 Kit Contents

Tube No.	Contents	Main Ingredients	Quantity
①	HPV^{19} Reaction Mix 1	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
②	HPV^{19} Reaction Mix 2	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
③	HPV^{19} Reaction Mix 3	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
④	HPV^{19} Reaction Mix 4	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
⑤	HPV^{19} Reaction Mix 5	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
⑥	HPV^{19} Reaction Mix 6	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
⑦	HPV^{19} Reaction Mix 7	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
⑧	HPV^{19} Reaction Mix 8	Primers, Probes, Mg^{2+}, dNTPs	1500 μL/tube ×1
/	HPV^{19} Enzyme Mix	Taq DNA Polymerase, Uracil-N-Glycosylase	180 μL/tube ×1
/	HPV^{19} Positive Control	Plasmid DNA	500 μL/tube ×1

The HPV detection information for each reaction mix is listed in Table 2.

Table 2 HPV detection information

Tube No.	Reagent Supplied	Fluorescent Signal		
		FAM	CY5	HEX/VIC
①	**HPV[19] Reaction Mix 1**	HPV16	HPV18	-
②	**HPV[19] Reaction Mix 2**	HPV58	HPV52	-
③	**HPV[19] Reaction Mix 3**	HPV33	HPV31	HPV26
④	**HPV[19] Reaction Mix 4**	HPV51	HPV82	HPV53
⑤	**HPV[19] Reaction Mix 5**	HPV45	HPV39	HPV73
⑥	**HPV[19] Reaction Mix 6**	HPV56	HPV35	HPV66
⑦	**HPV[19] Reaction Mix 7**	HPV59	HPV68	HPV70
⑧	**HPV[19] Reaction Mix 8**	HPV6	HPV11	Internal Control

Storage and Stability

The kit requires shipment on frozen ice packs. All contents of the kit should be stored immediately upon receipt at -20±5℃ and protected from light.

The shelf-life of the kit is twelve months. The maximal number of freeze-thaw cycle is five.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instruments are:
   Stratagene Mx3000P™, ABI7500, LightCycler480 SLAN-96S, or Rotor-Gene Q (72 wells).
2) Cervical exfoliated cells and urogenital tract secretion collection kit and sample vial.
3) DNA extraction kit: QIAamp DNA Mini Kit (Qiagen, Cat. No.: 51304) is recommended.
4) Spectrophotometer for measuring DNA concentration.
5) Mini centrifuge with rotor for centrifuge tubes.
6) Mini centrifuge with rotor for PCR tubes.
7) Vortexer.
8) Nuclease-free centrifuge tubes.
9) Nuclease-free PCR tubes and caps.
10) Adjustable pipettors and filtered pipette tips for handling DNA.
11) Tube racks.
12) Disposable powder-free gloves.
13) Sterile, nuclease-free water.

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use. Strictly follow the instruction during operation.
- Please check the compatible real-time PCR instruments prior to use.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals should use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid contact of the skin, eyes and mucous membranes with the chemicals. In case of contact, flush with water immediately.

- DO NOT pipet by mouth.

Decontamination and Disposal

- The kit contains positive control; strictly distinguish the positive control from other reagents to avoid contamination which may cause false positive.
- PCR amplification is extremely sensitive to cross-contamination. The flow of tubes, racks, pipets and other materials used should be from pre-amplification to post-amplification, and never backwards.
- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Use separate, dedicated pipettes and filtered pipette tips when handling samples and reagents to prevent exogenous DNA contamination to the reagents.
- Please pack the post-amplification tubes with two disposable gloves and discard properly. DO NOT open the post- amplification PCR tubes.
- All disposable materials are for one-time use. DO NOT reuse
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Instrument Setup

- Setup the reaction volume as 30 μL.
- For Stratagene Mx3000P™, if there's low net fluorescence signal (dR) but high background signal (R), please reduce the signal gain setting of instrument properly.
- For ABI instruments, please set up as follows: Reporter Dye: FAM, VIC and CY5; Quencher Dye: TAMRA; Passive Reference: NONE.
- For LightCycler480 I instrument, it's necessary to conduct fluorescence calibration prior to use. If there is fluorescence crossover on LightCycler480 II instrument, fluorescence calibration is also required. To run the assays on a LightCycler machine, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B79480.
- For Rotor-Gene instrument, after temperature profile editing, please set up as follows:
  ① Select "**Edit Gain**" to setup the appropriate gain setting for each channel according the instrument situation. For **Green** channel, please reduce the gain setting properly. (see Figure 1)
  ② select "**Gain Optimisation**", the "**Auto Gain Optimisation Setup**" window will open;
  ③ Click "**Perform Calibration Before 1st Acquisition**" and "**Optimise Acquiring**".
  ④ Click "**OK**", and "**Close**" to continue. Please see the following Figure 2, 3 and 4 for more details.

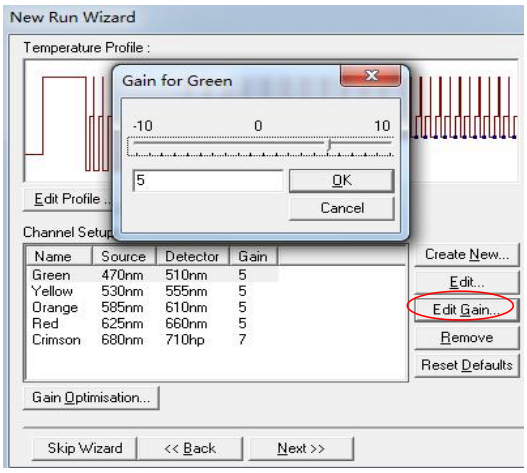

Figure 1

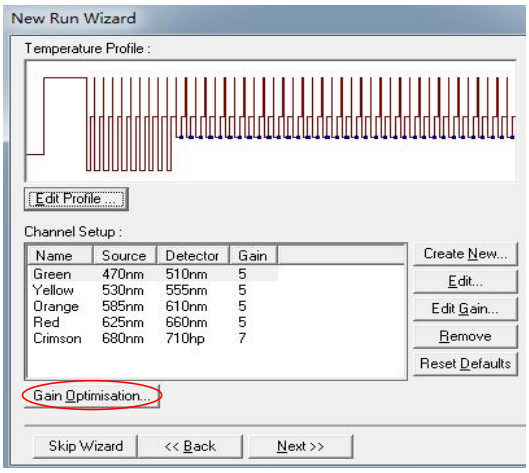

Figure 2

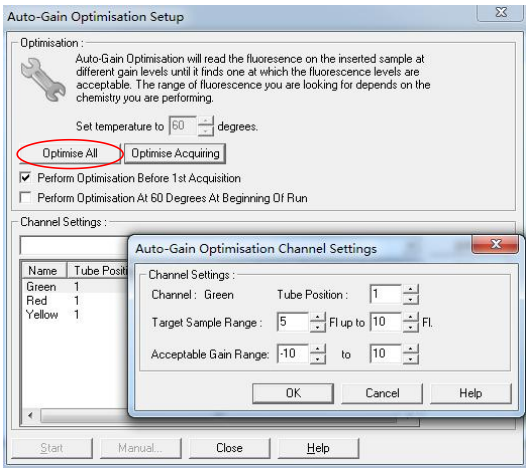

Figure 3

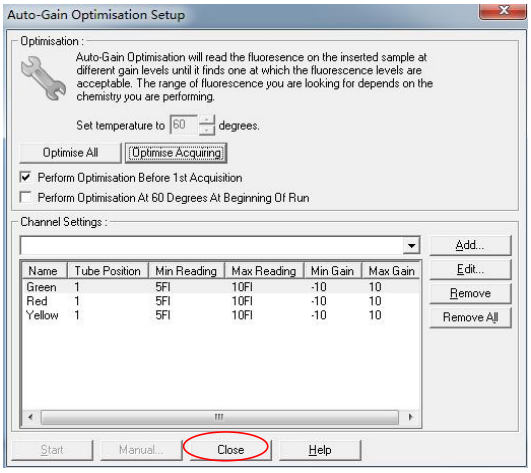

Figure 4

- Refer to the operation manual of real-time PCR instrument for detailed instructions.
- We recommend that for all PCR instruments in use, a fluorescence calibration should be conducted once a year.

Assay Procedure

1. DNA Extraction

The specimen material must be human DNA extracted from cervical exfoliated cells and urogenital tract secretion. The DNA extraction kit is not included in this kit.

1) For female cervical samples, use a scraper to scrape the exfoliated cells from the cervical lesions, insert the cervical scraper into the sterile sample collection vial.
2) Urogenital tract secretion samples include male urethra, female genital tract and urethra secretion.
   a) For male urethra, insert a tiny cotton swab into the urethral canal 2~4 cm, gently rotate the swab clockwise direction 3~5 times to collect the secretion, then place the sample in a sterile sample collection vial.
   b) For female genital tract, use a sterile saline cotton swab to remove extra secretion outside the cervix uteri, and insert a sterile brush or cotton swab into the endocervical canal, gently rotate the brush and swab clockwise direction 3~5 times to collect the cervical secretion, then place the sample in a sterile sample collection vial.
   c) For female urethra, use a sterile saline cotton swab to wash the urethra, and insert a sterile swab into the urethral canal 2 cm, gently rotate the swab clockwise direction 3~5 times to collect the secretion, then place the sample in a sterile sample collection vial.
3) The samples should be transported below 0℃ with ice bags and extracted to obtain DNA immediately. If the extracted DNA is not be used immediately, it should be stored at -20℃ for no more than 6 months.

2. HPV DNA Detection

1) Take the **HPV^{19} Reaction Mix 1~8**, **HPV^{19} Positive Control**, and **HPV^{19} Enzyme Mix** out of the kit from the freezer
2) Thaw **HPV^{19} Reaction Mix 1~8** and **HPV^{19} Positive Control (PC)** at room temperature. When the reagents are completely thawed, mix each reagent thoroughly by vortexing and centrifuge for 5~10 seconds to collect the contents at the bottom of the tube.
3) Centrifuge the **HPV^{19} Enzyme Mix** for 5~10 seconds prior to use.
4) Prepare sufficient HPV^{19} Master Mix 1~8 containing HPV^{19} Enzyme Mix and each Reaction Mix (HPV^{19} Reaction Mix 1~8, respectively) in separate sterile centrifuge tube according to the ratio in Table 3. Mix each HPV^{19} Master Mix thoroughly by vortexing and centrifuge for 5~10 seconds.

Table 3 HPV[19] Master Mix

Content	Volume per test
Reaction Mix (HPV[19] Reaction Mix 1~8, respectively)	25 μL
HPV[19] Enzyme Mix	0.3 μL
Total	**25.3 μL**

Note:

- *Every PCR run must contain one PC (Positive control) and one No Template Control (NTC).*
- *The prepared mixtures should be used immediately, avoid prolonged storage.*
- *Due to the viscosity of the enzyme mix, pipet slowly to ensure all mix is completely dispensed from the tip.*
- *Pipet enzyme mix by placing the pipet tip just under the liquid surface to avoid the tip being coated in excess enzyme.*

5) Take out the sample DNA and NTC.

Note: *sterile water, 1×TE buffer or normal human genomic DNA solution could be used as NTC.*

6) Prepare 8 PCR tubes for NTC: Dispense 25.3 μL of each HPV[19] Master Mix 1~8 to each PCR tube respectively, then add 4.7 μL NTC to each PCR tube. Cap the PCR tubes.

7) Prepare 8 PCR tubes for each sample: Dispense 25.3 μL of each HPV[19] Master Mix 1~8 to each PCR tube respectively, then add 4.7 μL each sample DNA to each PCR tube. Cap the PCR tubes.

8) Prepare 8 PCR tubes for PC: Dispense 25.3 μL of each HPV[19] Master Mix 1~8 to each PCR tube respectively, then add 4.7 μL PC to each PCR tube of PC strip. Cap the PCR tubes.

Note: *Suggest following below adding order: NTC → samples → PC, and use filtered tips for all pipetting steps to avoid cross-contamination.*

9) Place the PCR strips into the real-time PCR instrument. A recommended plate layout is shown in Table 4

Table 4 Recommended PCR Plate Layout

Tube	1	2	3	…	8	9	10	11	12
①	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC
②	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC
③	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC
④	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC
⑤	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC
⑥	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC
⑦	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC
⑧	Sample1	Sample2	Sample3	…	Sample8	Sample9	Sample10	PC	NTC

10) Setup the PCR Protocol using the cycling parameters in Table 5.

Table 5 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	50℃	2 min	/
		95℃	5 min	/
2	10	95℃	25 s	/
		60℃	20 s	/
		72℃	20 s	/
3	31	95℃	25 s	/
		60℃	35 s	FAM, HEX/VIC and CY5
		72℃	20 s	/

11) Start the PCR run immediately.

12) When the PCR run is finished, analyze the data according to the “Results Interpretation” procedures.

3. Results Interpretation

Before sample data analysis, the following items should be checked:

1) For NTC: The FAM & CY5 Ct values in Tubes ①~⑧and HEX/VIC Ct values in Tubes ③~⑦ should be ≥**31**. If not, the data is *INVALID*. The sample should be retested.
2) For **HPV[19] Positive Control**: The FAM & CY5 Ct values in tubes ①~⑧ and HEX/VIC Ct values in tubes ③~⑦ should be ≤ **23**, and HEX/VIC Ct values in Tube ⑧ should be ≤ **29**.
3) For HEX/VIC signal in Tube ⑧ of each sample: The HEX/VIC Ct value in Tube ⑧ should be ≤ 29. If HEX/VIC Ct value > 29, this indicates the presence of PCR inhibitors or insufficient DNA, the sample should be retested with increased or re-extracted DNA. But if any of the FAM/CY5 Ct value in Tubes ①~⑧ or any of the HEX/VIC Ct value in Tubes ③~⑦ Ct value is ≤ 27, the sample is determined as **HPV DNA positive**.

Analyze the result for each sample:

4) Analyze the FAM and CY5 signals in Tubes ①~⑧, and HEX/VIC signal in Tubes ③~⑦.
   a) **HPV DNA Positive**: if any of the FAM and CY5 signals in Tubes ①~⑧, or any of the HEX/VIC signal in Tubes ③~⑦ is S-curve and Ct value is ≤ **27**, the sample is determined as HPV DNA positive. Please see the detailed result interpretation information in Table 5.
   b) **HPV DNA Negative:** if the FAM & CY5 Ct values in Tubes ①~⑧ and HEX/VIC Ct values in Tubes ③~⑦ all are > **27**, the sample is determined as HPV DNA negative or below the detection limit (LOD) of the kit.

Table 5 Result Determination

Tube No.	FAM Signal	CY5 Signal	HEX/VIC Signal
	S-Curve and Ct ≤27	S-Curve and Ct ≤27	S-Curve and Ct ≤27
①	HPV16 DNA positive	HPV18 DNA positive	-
②	HPV58 DNA positive	HPV52 DNA positive	-
③	HPV33 DNA positive	HPV31 DNA positive	HPV26 DNA positive
④	HPV51 DNA positive	HPV82 DNA positive	HPV53 DNA positive
⑤	HPV45 DNA positive	HPV39 DNA positive	HPV73 DNA positive
⑥	HPV56 DNA positive	HPV35 DNA positive	HPV66 DNA positive
⑦	HPV59 DNA positive	HPV68 DNA positive	HPV70 DNA positive
⑧	HPV6 DNA positive	HPV11 DNA positive	-

5) The sample may contain two or more positive HPV DNA simultaneously.

Performance Characteristics

The performance characteristics of this kit were validated on Stratagene Mx3000P™, ABI7500, LightCycler480, SLAN-96S and Rotor-Gene Q (72 wells).

1) Limit of Detection:
   For HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82, the kit allows detection of 100 copies HPV DNA per reaction.
2) Specificity:
   Specificity of the kit was established by testing negative reference controls, the test gave negative results and negative concordance rate was 100%.
3) Accuracy:
   Accuracy of the kit was established by testing positive reference controls, the test gave positive results and positive concordance was 100%.
4) Precision:
   Precision of the kit was established by performing a certain HPV DNA positive reference control for 10 repeats; all the controls can be detected with positive FAM, CY5 and HEX/VIC signals and the CV of Ct values is less than 5%.

5) Cross-reactivity:

The kit has no cross-reactivity with *chlamydia trachomatis*, *ureaplasma urealyticum*, *neisseria gonorrhoeae*, *herpes simplex virus*, *syphilis*, *mycoplasma hominis*, *monilia albican*, and *trichomonas vaginalis* pathogen DNA. The kit also has no cross-reactivity with other HPV types DNA (HPV40, 42, 43, 44, 54, 61, 72, 81, and 83).

6) Interfering substance:

Several potential interfering substances: potassium permanganate residue (≤ 1.0%), soda residue (≤ 8%), hemoglobin (≤ 30 mg/mL), leukocyte (≤ 4.0×10^6 CFU/mL), miconazole nitrate acid (≤ 100 mg/mL), nonoxynol suppository (≤ 100 mg/mL), vaginal lubricant (≤ 10%) and various abnormal types of cervical secretions specimens were evaluated. The results show these interfering substances would not interfere with the test result.

Limitations

1) The kit is to be used only by personnel specially trained with PCR techniques.
2) The results can be used to assist clinical diagnosis, combining with other clinical and laboratory findings.
3) The kit has been validated for use with cervical exfoliated cells and urogenital tract secretion.
4) The kit can only detect the 21 HPV DNA listed in the instruction.
5) Reliable results are dependent on proper sample processing, transport, and storage.
6) The sample containing degraded DNA may affect the ability of the test to detect HPV DNA.
7) Samples with negative result may harbor HPV DNA not detected by this assay.

References

1) Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide, J Pathol, 1999; 189(1):50-53.
2) Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ, Epidemiologic classification of human papillomavirus types associated with cervical cancer, N. Engl. J. Med. 2003; 348 (6): 518–27.

Symbols

	Manufacturer	REF	Catalogue Number
LOT	Batch Code		Use-by Date
Σ	Contains Sufficient for <n> Tests		Temperature Limit
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care
	Keep Away from Sunlight		