CE IVD

AmoyDx® High-risk Human Papillomavirus (HPV) Detection Kit

Detection of 19 High-risk Human Papillomavirus

Instruction for Use

REF 8.01.25802X048E	48 tests	For Stratagene Mx3000P™, ABI 7500, LightCycler 480, Bio-Rad CFX96, SLAN-96S, Rotor Gene Q/6000 (72 wells)

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Version: B2.4
July 2019

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® High-risk Human Papillomavirus (HPV) Detection Kit is a real-time PCR assay for qualitative detection of 19 high-risk HPV DNA (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82) 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 *in vitro* diagnostic use, 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 **HPV^{18} Reaction Mix, HPV^{18} Enzyme Mix** and **HPV^{18} Positive Control.**

1) The **HPV^{18} Reaction Mix** includes a HPV DNA detection system and an internal control system. It contains primers and CY5-labeled probes specific for HPV 16/18 DNA, FAM-labeled probes specific for other 17 high-risk HPV types DNA, HEX-labeled probe specific for internal control. The internal control system is designed to detect a housekeeping gene as reference gene to reveal the presence of inhibitors and monitor the accuracy of experimental operation, which may lead to false negative results.
2) The **HPV^{18} 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.
3) The H**PV^{18} Positive Control** contains recombinant gene with HPV 18 and 26 types' plasmid DNA.

Kit contents

The kit contains the following materials (Table 1).

Table 1 Kit Contents

Tube No.	Content	Main Ingredient	Quantity	Channel
①	**HPV^{18} Reaction Mix**	Primers, Probes, dNTPs	1800 μL/tube ×1	FAM, HEX/VIC, CY5
②	**HPV^{18} Enzyme Mix**	Taq DNA Polymerase, Uracil-N-Glycosylase	30 μL/tube ×1	\
③	**HPV^{18} Positive Control**	Plasmid DNA	100 μL/tube ×1	\

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 recommend maximum freeze-thaw cycle is five cycles.

Additional Reagents and Equipment Required but Not Supplied

1) Compatible PCR instruments are:

   Stratagene Mx3000P™, ABI 7500, LightCycler 480, Bio-Rad CFX96, SLAN-96S, or Rotor Gene Q/6000 (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) Nuclease-free centrifuge tubes.
8) Nuclease-free PCR tubs and caps.
9) Adjustable pipettors and filtered pipette tips for handling DNA.
10) Tube racks.
11) Disposable powder-free gloves.
12) Sterile, nuclease-free water.

Precautions and Handling Requirements

For *in vitro* diagnostic use.

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, and 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 can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Avoid skin, eyes and mucous membranes contact 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.
- Using 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 40 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.

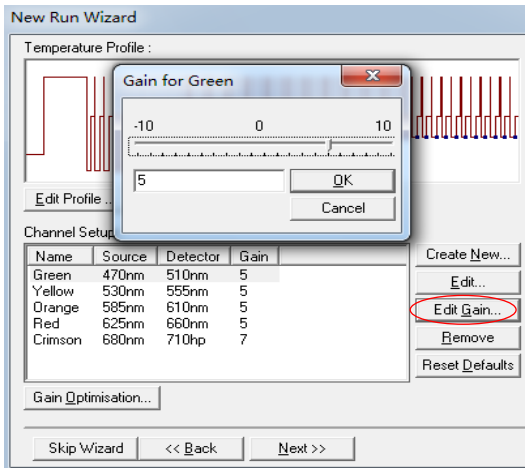

Figure 1

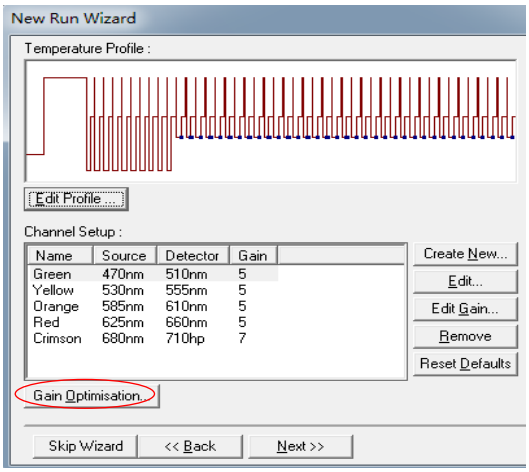

Figure 2

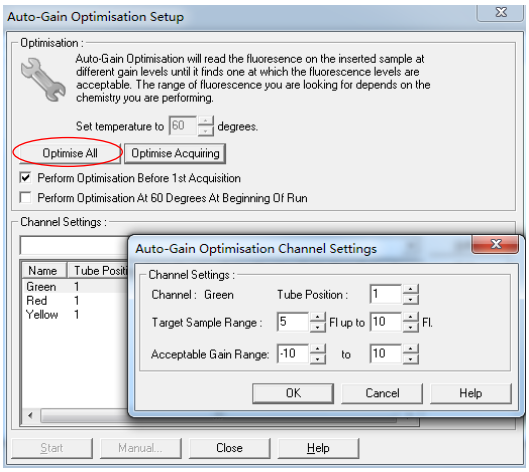

Figure 3

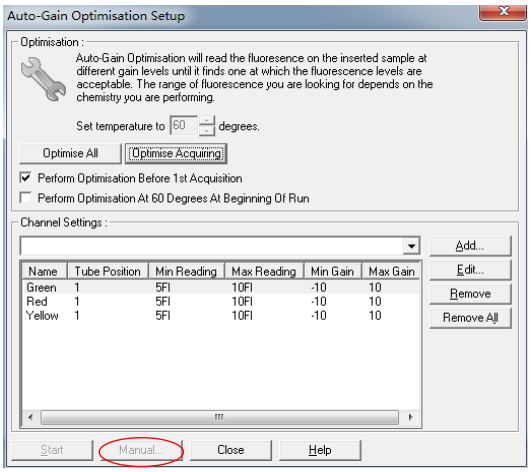

Figure 4

- Refer to the 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.

Assay Procedure

1. DNA Extraction

The specimen must be human genomic 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) Thaw **HPV^{18} Reaction Mix** and **HPV^{18} Positive Control** at room temperature. When the reagents completely thawed, mix the reagents by inverting the tube 10 times and centrifuge briefly to collect the contents at the bottom of the tube.
2) Centrifuge the **HPV^{18} Enzyme Mix** prior to use.
3) According to the ratio of 35 μL **HPV^{18} Reaction Mix** to 0.48 μL **HPV^{18} Enzyme Mix** per sample, to prepare HPV Master Mix, transfer the appropriate amount of Reaction Mix and Enzyme Mix into a sterile tube. Mix the HPV Master Mix thoroughly by gently pipetting up and down more than 10 times and centrifuge briefly.

   Note:
   - *Every PCR run must contain one PC (Positive control) and one NTC (No template control).*
   - *Do not store user-prepared mixes, use immediately.*
   - *Since the enzyme mix is viscous, please pay attention to the centrifugation and pipetting process.*
   - *Minimize the contact interface between the pipette tip and enzyme mix to avoid adding excess enzyme.*
   - *Avoid vortexing solutions with enzyme mix.*
4) Take out the sample DNA and nuclease-free water for NTC.
5) Prepare sufficient PCR tubes for samples, PC and NTC. Transfer 35 μL the HPV Master Mix into the appropriate PCR tubes.
6) Add 5 μL NTC , 5 μL sample DNA or 5 μL **HPV^{18} Positive Control** to the appropriate PCR tubes. Cap the PCR tubes.
   - NTC: sterile water, 1×TE buffer or normal human genomic DNA solution could be used as NTC.
7) Briefly centrifuge the PCR strips to collect all liquid at the bottom of each PCR tube.
8) Place the PCR strip tubes into the real-time PCR instrument.
9) Setup the PCR Protocol using the cycling parameters in Table 2.
10) Start the PCR run immediately.
11) When the PCR run finished, analyze the data according to the "Results Interpretation" procedures.

Table 2 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	50℃	2 min	/
		95℃	5 min	/
2	10	95℃	5 s	/
		40℃	30s	/
		72℃	30s	/
3	35	95℃	5s	/
		60℃	35s	FAM, HEX/VIC and CY5
		72℃	30s	/

3. Results Interpretation

Before data analysis, the following items should be checked:

1) For NTC: The CY5 and FAM Ct values should be ≥**35**. If not, the data is *INVALID*. The sample should be retested.
2) For **HPV[18] Positive Control**: The CY5 and FAM Ct values should be ≤ **20**, and HEX/VIC Ct value should be ≤ **29**.
3) For HEX/VIC signal of each sample: The HEX/VIC Ct value 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 the FAM or CY5 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 for each sample (according to Table 3):
   a) **HPV 16/18 DNA Positive**: if the sample CY5 signal is S-curve and CY5 Ct value is less than or equal to the critical value (Ct ≤ **27**), the sample is determined as HPV 16/18 DNA positive.
   b) **Other 17 high-risk HPV DNA Positive**: if the sample FAM signal is S-curve and FAM Ct value is less than or equal to the critical value (Ct ≤ **27**), the sample is determined as Other 17 high-risk HPV DNA positive.
   c) **HPV DNA Negative:** if the sample FAM and CY5 signals are not S-curve or both of Ct values are greater than the critical value (Ct > **27**), the sample is determined as HPV DNA negative or below the detection limit (LOD) of the kit.
5) The sample may contain two or more positive HPV DNA simultaneously.

Table 3 Result Determination

Sample type	CY5	FAM	HEX/VIC	Result interpretation
1	S-Curve and Ct ≤27	S-Curve and Ct ≤27	S-Curve or not	HPV 16/18 DNA positive and other 17 high-risk HPV DNA positive simultaneously.
	S-Curve and Ct ≤27	No S-Curve or Ct >27	S-Curve or not	HPV 16/18 DNA positive.
	No S-Curve or Ct >27	S-Curve and Ct ≤27	S-Curve or not	Other 17 high-risk HPV DNA positive.
2	No S-Curve or Ct >27	No S-Curve or Ct >27	Ct ≤29	HPV DNA Negative or below the LOD.
3	No S-Curve or Ct >27	No S-Curve or Ct >27	Ct >29	Suggest retest the sample.

Performance Characteristics

The performance characteristics of this kit were validated on Stratagene Mx3000P™, ABI7500, LightCycler 480, Bio-Rad CFX96, SLAN-96S, and Rotor Gene Q/6000 (72 wells).

1) Physical Performance:
   Kit appearance is complete and clearly marked without leakage; after melted, reagent solution is clarify without turbidity or precipitate.
2) Limit of Detection:
   a. For HPV16 type, the kit allows detection of 1000 copies HPV DNA per reaction.
   b. For HPV45, 53, 59, 73 types, the kit allows detection of 50 copies HPV DNA per reaction.
   c. For other HPV types, the kit allows detection of 500 copies HPV DNA per reaction.
3) Accuracy:
   Accuracy of the kit was established by testing 20 HPV DNA positive reference controls and 5 negative reference controls, all the detection concordance rate are 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 CY5 and FAM 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 (HPV6, 11, 40, 42, 43, 44, 54, 61, 72, 81, and 83).

6) Interference factor:

Several interference 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 in this study. The result show these interference 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 19 high-risk 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

EC REP	Authorized Representative in the European Community	IVD	In Vitro Diagnostic Medical Device
	Manufacturer	REF	Catalogue Number
LOT	Batch Code		Use By
Σ	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care