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

Detection of 19 High-risk Human Papillomavirus

Instruction for Use

Instruction Version: B1.0
Revision Date: January 2015

Store at -20±5°C
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.

The AmoyDx® High-risk Human Papillomavirus (HPV) Detection Kit is highly sensitive real-time PCR based test for detection of 19 high-risk human papillomavirus: HPV16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82. The purpose of the kit is designed for cervical cancer screening, early diagnostics and treatment.

Intended Use

The AmoyDx® High-risk Human Papillomavirus (HPV) Detection Kit is a qualitative real-time PCR-based in vitro test designed to accurately identify 19 high-risk HPV DNA in intact human cells. The assay is designed and optimized to detect the following HPV types: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, and 82. The used HPV DNA is extracted from cervical exfoliated cells and urogenital tract secretion.

Kit contents

This kit contains sufficient reagents to carry out 48 tests (Table 1), and additional HPV19 Positive Control for positive control reactions.

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Reagents Supplied</th>
<th>Volume (μL)</th>
<th>Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HPV19 Reaction Mix</td>
<td>1800</td>
<td>FAM, HEX/VIC, CY5</td>
</tr>
<tr>
<td>2</td>
<td>HPV19 Enzyme Mix</td>
<td>30</td>
<td>\</td>
</tr>
<tr>
<td>3</td>
<td>HPV19 Positive Control</td>
<td>100</td>
<td>\</td>
</tr>
</tbody>
</table>

Equipment and Reagents Not Supplied With Kit

1. Compatible PCR instruments are:
   - Stratagene Mx3000P™, ABI7500, LightCycler 480, Bio-Rad CFX96, and SLAN®-96S/96P.
   - To run the assay on a LightCycler machine, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B-79480. If the fluorescence crossover occurs in the LightCycler480 II instrument, the fluorescence calibration is also needed prior to the operation.
   - 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.
   - We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

2. Cervical exfoliated cells and urogenital tract secretion collection kit and sample vial.

3. HPV DNA extraction kit (recommend use of DNA extraction kit: AmoyDx® Cell DNA Kit, Cat No. ADx-
Sterile, nuclease-free tubes.
5. Dedicated pipettes and filtered pipette tips for handling DNA.
6. Sterile, nuclease-free H2O.

Shipping and Storage
The kit requires cold-chain-transportation with ice bags. All contents of the kit should be stored immediately upon receipt at -20±5°C. Avoid unnecessary freezing and thawing of the kit contents. Do not use the reagent after five freeze-thaw cycles. Once opened, this reagent is stable at -20±5°C until the expiry.

Stability
The shelf-life of the kit is twelve months when stored under the recommended conditions and in the original packaging. Do not use the kit after the stated expiry date.

Specimen Material
Human HPV DNA must be extracted from cervical exfoliated cells and urogenital tract secretion, and stored at -20°C prior to use.
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°C with ice bags and extracted HPV DNA immediately. If not be used immediately, it should be stored at -20°C for no more than 6 months.

Technological Principles
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 novel fluorescence probes. A non-rivalry internal control is added in the HPV DNA detection system to ensure proper PCR procedure.

Protocol
1. The reaction mix contains the reaction buffer, dNTPs, specific oligos and probes.
2. The HPV19 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.
3. The HPV19 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 HPV19 Positive Control contains a recombinant gene with HPV 18 and 26 types’ plasmid DNA.

Experimental Procedure

1. Thaw HPV19 Reaction Mix and HPV19 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 HPV19 Enzyme Mix prior to use.

3. According to the ratio of 35 μL HPV19 Reaction Mix to 0.48 μL HPV19 Enzyme Mix per sample, transfer the appropriate amount of Reaction Mix and Enzyme Mix into a sterile tube.

   Note:
   - The volumes given for each reaction mix have been optimized and validated. Changing volumes of any reagent may result in a loss of performance.
   - 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.
   - NTC: sterile water, 1×TE buffer or normal human genomic DNA solution could be used as NTC.

4. Mix the solution thoroughly by gently pipetting up and down more than 10 times.

   Note: avoid vortexing solutions with HPV19 enzyme mix.

5. Centrifuge briefly.

6. Transfer 35 μL the above master mix into the appropriate PCR tubes.

7. Add 5 μL sample HPV DNA, 5 μL HPV19 Positive Control (PC) or 5 μL ddH2O (no-template control, NTC) to the appropriate PCR tubes.

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

8. Seal the PCR tubes.

9. Spin the PCR tubes gently to collect the reagents at the bottom of tubes and centrifuge briefly if there are droplets on the lid and wall of PCR tubes.

   Note: this spin step is essential for proper mixing of the reagents.

10. Place the PCR tubes into the real-time PCR instrument.

    Note: place the PCR tubes into the real-time PCR instrument and start to run immediately. If not, please store the PCR tubes at 4°C for no more than 6 hours.

11. Carry out real-time PCR using the cycling conditions described in Table 2.

    Note:
    - The reaction volume is 40 μL per well (35 μL reagents plus 5 μL template).
    - Please pack the post-PCR tubes with two disposable gloves and discard properly. Do NOT open the post-PCR tubes to avoid contamination.
Table 2 Cycling Parameters

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50℃</td>
<td>2min</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>95℃</td>
<td>5min</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>95℃</td>
<td>5s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40℃</td>
<td>30s</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>72℃</td>
<td>30s</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>95℃</td>
<td>5s</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>60℃</td>
<td>35s</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72℃</td>
<td>30s</td>
<td></td>
</tr>
</tbody>
</table>

(☆ Data collection of FAM, HEX/VIC and CY5)

Sample Data Analysis

1. The CY5 signals of HPV DNA detection system indicate HPV 16/18 DNA present and the FAM signals indicate the other 17 high-risk HPV DNA present. The HEX/VIC signals indicate the internal control status.

2. Ensure the calibration fluorescence is unselected, and select single detection for each tube accordingly. It is necessary to choose the reaction wells for positive control, no-template control and sample simultaneously. Then users could adjust the threshold of amplification curve, and obtain the Ct value of reaction mix group.

3. The threshold at which the signal is detected above background fluorescence is called the Cycle threshold (Ct). The Ct values used to determine if a sample is positive or negative are based on extensive validation. If the Ct value falls within the appointed range, the sample is classed as positive. If the Ct value is outside the appointed range, the sample is classed as negative or below the detection limit of the kit.

4. Assess the NTC signals to ensure that there are no S-Curves. If the FAM or CY5 signal of NTC has positive amplification, the data must be discarded as there is contamination. If the HEX/VIC signal of NTC occasionally rises, further analysis could be carried out.

5. The HPV16 Positive Control CY5 and FAM Ct value should be less than or equal to 20 (Ct ≤ 20), HEX/VIC signal should be less than or equal to 29 (Ct ≤ 29), but variation may occur due to different threshold settings on different instruments.

6. Assess the HEX/VIC signal for each sample, to check the internal control status:
   1) The HEX/VIC signal should be S-curve and Ct value should be less than or equal to 29 (Ct ≤ 29).
   2) If the internal control assay fails, it shows that the DNA template contains PCR inhibitors or be insufficient, indicating that the DNA needs to be re-extracted or increase the DNA amount. If FAM or CY5 signal is positive, the sample is classified as HPV DNA positive.

7. The HPV DNA Negative: if the sample FAM and CY5 signals are not S-curve and Ct values are greater than the critical value (Ct > 27), the sample is classified as HPV DNA negative or below the detection limit of the kit.
8. The sample may contain two or more positive HPV DNA simultaneously.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>CY5</th>
<th>FAM</th>
<th>HEX/VIC</th>
<th>Result interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S-Curve and Ct ≤27</td>
<td>S-Curve and Ct ≤27</td>
<td>S-Curve or not</td>
<td>HPV 16/18 DNA positive and other 17 high-risk HPV DNA positive simultaneously.</td>
</tr>
<tr>
<td></td>
<td>S-Curve and Ct ≤27</td>
<td>No S-Curve or Ct &gt;27</td>
<td>S-Curve or not</td>
<td>HPV 16/18 DNA positive.</td>
</tr>
<tr>
<td></td>
<td>No S-Curve or Ct &gt;27</td>
<td>S-Curve and Ct ≤27</td>
<td>S-Curve or not</td>
<td>Other 17 high-risk HPV DNA positive.</td>
</tr>
<tr>
<td>2</td>
<td>No S-Curve or Ct &gt;27</td>
<td>No S-Curve or Ct &gt;27</td>
<td>Ct ≤29</td>
<td>HPV DNA Negative or below the LOD.</td>
</tr>
<tr>
<td>3</td>
<td>No S-Curve or Ct &gt;27</td>
<td>No S-Curve or Ct &gt;27</td>
<td>Ct &gt;29</td>
<td>Suggest rerun the sample.</td>
</tr>
</tbody>
</table>

Performance Characteristics

The performance characteristics of this kit were validated on Stratagene Mx3000P™, ABI7500, LightCycler 480, Bio-Rad CFX96, and SLAN® 96S/96P.

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:
   1) For HPV16 type, the kit allows detection of 1000 copies HPV DNA per reaction.
   2) For HPV45, 53, 59, 73 types, the kit allows detection of 50 copies HPV DNA per reaction.
   3) For other HPV types, the kit allows detection of 500 copies HPV DNA per reaction.

3. Productivity: the kit can be used to analysis 48 samples maximum. (see Table 4)

<table>
<thead>
<tr>
<th>PCR Run(s)</th>
<th>Control Qty</th>
<th>Total samples can be detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 run</td>
<td>2 (2 controls/run * 1)</td>
<td>48</td>
</tr>
<tr>
<td>2 runs</td>
<td>4 (2 controls/run * 2)</td>
<td>46</td>
</tr>
<tr>
<td>3 runs</td>
<td>6 (2 controls/run * 3)</td>
<td>44</td>
</tr>
<tr>
<td>4 runs</td>
<td>8 (2 controls/run * 4)</td>
<td>42</td>
</tr>
<tr>
<td>5 runs</td>
<td>10 (2 controls/run * 5)</td>
<td>40</td>
</tr>
</tbody>
</table>

4. Accuracy:
   - Accuracy of the kit was established by testing 20 HPV DNA positive reference controls, 5 negative reference controls and 11 specific reference controls, all the detection concordance rate are 100%.

5. 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%.

6. Cross-reactivity:
   - The kit has no cross-reactivity with chlamydia trachomatis, ureaplasma urealyticum, neisseria gonorrhoeae, herpes simplex virus, syphilis and other similar pathogen DNA.
Warnings and Precautions

1. Please read the instruction carefully and become familiar with all components of the kit prior to use.
2. The product specified above does not contain any virus, reagent by-product of the same or metabolic by-product of Hepatitis A, B, C, D or HIV.
3. Do not exchange and mix up the kit contents with different batches.
4. The kit and its contents cannot be resold or modified for resale without the written approval of manufacturer.
5. Using other sources of reagents is not recommended. Strictly distinguish the reagents from mixed standard to avoid contamination. Otherwise, false positive may be produced.
6. Do the experiments with attention to prevent exogenous DNA contamination to reagents. It is recommended that users have separate, dedicated pipettes and filtered pipette tips to add DNA template during the preparation of reagents.
7. To optimize the activity and performance, mixtures should always be protected from light to avoid photo bleaching.
8. All the chemicals are potential hazard, only trained professionals could use this kit. Please wear suitable lab coat and disposable gloves. The used kit should be disposed of properly.
9. The kits can’t detect HPV DNA other than the 19 HPV types indicated in this instruction.
10. Wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid.
11. The flow of tubes, racks, pipets and other equipment used should be from pre-amplification to post-amplification, and never backwards.

Notes

1. Symbol for "AUTHORISED REPRESENTATIVE IN THE EUROPEAN COMMUNITY"
2. Symbol for "IN VITRO DIAGNOSTIC MEDICAL DEVICE"
3. Symbol for "KEEP DRY"
4. Symbol for "THIS WAY UP"
5. Symbol for "FRAGILE , HANDLE WITH CARE"

Information of European Authorised Representative

Wellkang Ltd t/a Wellkang Tech Consulting Suite B, 29 Harley Street, London W1G 9QR United Kingdom

References