AmoyDx® HER2 Mutation Detection Kit

Detection of five mutations in HER2 gene

Instruction for Use

For Research Use Only

Instruction Version: B1.0
Revision Date: January 2014

Store at -20±5℃
Background

Human epidermal growth factor receptor 2 (HER2), also known as Neu, ErbB-2, CD340 (cluster of differentiation 340) or p185, is encoded by a proto-oncogene ERBB2 which located at the long arm of human chromosome 17 (17q12). Activating mutations in the tyrosine kinase domain of HER2 have been described in a subset of lung adenocarcinomas (ADCs) and are mutually exclusive with EGFR/KRAS/ALK driver mutations. HER2 mutations, consisting of in-frame insertions in exon 20, have been identified in approximately 2~4% of NSCLC patients. These insertions cause activation of downstream HER2-pathway components such as AKT and MEK.

Studies have shown that NSCLC patients harboring a HER2 exon 20 insertion are sensitive to trastuzumab (marketed as Herceptin) and the irreversible dual EGFR and HER2 TKIs, lapatinib, neratinib, and afatinib. However, the presence of this mutation is associated with primary resistance to the first generation EGFR TKIs, erlotinib and gefitinib. These studies highlighted the importance of screening for HER2 mutations in NSCLC and suggest that HER2-positive patients may be responsive to HER2-targeted therapy.

Intended Use

The AmoyDx® HER2 Mutation Detection Kit is highly selective and sensitive in detection of five mutations in the HER2 gene (Table 1). Our company’s patented technology allows detection of 1% mutant DNA in a background of 99% normal DNA at 10 ng sample DNA amount, while ensuring that false negatives are minimized. This kit is intended for research use only.

Table 1. Details of five somatic mutations in HER2 gene

<table>
<thead>
<tr>
<th>Name</th>
<th>Mutation</th>
<th>Base Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2-M1</td>
<td>A775_G776insYVMA</td>
<td>2325_2326 ins12 (ta tgtgatggtc)</td>
</tr>
<tr>
<td>HER2-M2</td>
<td>A775_G776insYVMA</td>
<td>2324_2325 ins12 (atactgtgatggc)</td>
</tr>
<tr>
<td>HER2-M3</td>
<td>G776&gt;VC</td>
<td>2326_2327 ins3 (tgt)</td>
</tr>
<tr>
<td>HER2-M4</td>
<td>P780_Y781insGSP</td>
<td>2339_2340 ins9 (tggctcccc)</td>
</tr>
<tr>
<td>HER2-M5</td>
<td>M774_A775insAYVM</td>
<td>2322_2323 ins12 (gcatactgtag)</td>
</tr>
</tbody>
</table>

Kit Contents

This kit contains sufficient reagents to carry out 24 tests (Table 2), and additional HER2 Positive Control DNA for positive control reactions.

Table 2. Kit Contents

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Reagents Supplied</th>
<th>Volume (μL)</th>
<th>Tube (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>①</td>
<td>HER2 Reaction Mix①</td>
<td>1100</td>
<td>1</td>
</tr>
<tr>
<td>②</td>
<td>HER2 Reaction Mix②</td>
<td>1100</td>
<td>1</td>
</tr>
<tr>
<td>③</td>
<td>HER2 Reaction Mix③</td>
<td>1100</td>
<td>1</td>
</tr>
<tr>
<td>④</td>
<td>HER2 Enzyme Mix</td>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>⑤</td>
<td>HER2 Positive Control</td>
<td>250</td>
<td>1</td>
</tr>
</tbody>
</table>

Equipment and Reagents Not Supplied With Kit

1. Compatible PCR instruments are:

Stratagene Mx3000P™/Mx3005P™, ABI7300, ABI7500, LightCycler480 I and II, BioRad-CFX96.

(1) This kit is compatible with LightCycler480 II instrument. Fluorescence calibration is required for LightCycler480 I instrument. If the fluorescence crossover occurs in the LightCycler480 II instrument,
the fluorescence calibration is also needed prior to the operation. To run the assay on a LightCycler machine, please use the Roche 480 adaptor, available from BIOplastics, Cat No. B79480.

(2) For ABI instruments please set up as follows: Reporter Dye: FAM; Quencher Dye: TAMRA; Passive Reference: NONE.

(3) For Stratagene Mx3000P™/Mx3005P™, if there’s low net fluorescence signal (dR) but high background signal (R), please reduce the signal gain setting of instrument properly.

(4) We recommend that all PCR instruments in use should be conducted fluorescence calibration once a year.

2. Sterile, nuclease-free tubes.
3. Dedicated pipette and filtered pipette tips for handling DNA samples.
4. Sterile, nuclease-free H₂O.

Shipping and Storage
The kit requires cold-chain-transportation. All contents of the kit should be stored immediately at -20±5°C upon receipt. Avoid unnecessary freezing and thawing of the kit contents.

Stability
The shelf-life of the kit is eight 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 genomic DNA must be extracted from tissue, formalin-fixed paraffin-embedded (FFPE) samples or body fluid. The FFPE samples should be made and stored following proper procedures. The slides should preferably be less than two years. Before extraction of DNA, it is very important to make sure that there is at least 5% tumor cells in the FFPE tissue samples. If the extracted DNA is not used immediately, it should be stored at -20±5°C for no more than 6 months. High quality DNA is essential and we recommend use of DNA extraction kit (AmoyDx® FFPE DNA Kit, Cat No. ADx-FF01, for paraffin embedded specimens; AmoyDx® Tissue DNA Kit, Cat No. ADx-TI01, for tissue and pleural effusion specimens). The OD value of DNA samples should be measured using the spectrophotometer after extraction. Make sure A₂₆₀/A₂₈₀ value between 1.8 and 2.0.

Technological Principles
The kit uses novel, proprietary primers and probes in a real-time PCR assay to detect five HER2 mutations in human genomic DNA. The mutant HER2 gene is amplified by the specific primers, and detected by the novel probes.

Protocol
1. The reaction mix tubes contain the reaction buffer, dNTPs, specific oligos and probes.
2. The HER2 Reaction Mix①/② includes a mutation detection system used to detect the mutation status of HER2 gene (positive or negative). The HER2 Reaction Mix③ contains external control to assess the DNA quality, that is, to detect the presence of inhibitors, which may lead to false negative results.
3. The HER2 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 HER2 Positive Control contains an HER2 gene with 5 mutations and normal human genomic DNA.
5. 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 (see below), the sample is classed as mutation positive. If the Ct value is outside the appointed range, the sample is classed as negative or below the detection limit of the kit.
6. The mutation assay for sample and control must be analyzed within the same PCR run to avoid run-to-run
variations in threshold settings. It is recommended that the HER2 Positive Control (PC) should be analyzed during each PCR run, along with no-template controls (NTC).

Experimental Procedure

1. Thaw HER2 Reaction Mix①~③ and HER2 Positive Control at room temperature.
2. Briefly centrifuge HER2 Enzyme Mix, HER2 Reaction Mix①~③ and HER2 Positive Control prior to use.
3. According to the ratio of 0.3 μL HER2 Enzyme Mix to 35 μL HER2 Reaction Mix①~③ per sample, transfer the appropriate amount of HER2 Enzyme Mix and HER2 Reaction Mix①~③ into a sterile tube.
4. Mix the solution thoroughly by gently pipetting it up and down.
5. Centrifuge briefly.
6. Transfer 35 μL of the above mixed solution into the appropriate PCR tubes.
7. Add 5 μL sample DNA (2~3 ng/µL), 5 μL HER2 Positive Control (PC), or 5 μL ddH2O (NTC) to the appropriate PCR tubes.

Note:
- We recommend use of TE (pH = 8.0) for extracted DNA dilution. Suggest using at least 5 μL DNA for dilution within ten-fold series, to ensure the accuracy of final concentration.
- Since the Enzyme Mix is viscous, please pay attention to the centrifugation and pipetting process.
- Minimize the contact interface between the pipette tip and the Enzyme Mix to avoid adding excess enzyme.
- Avoid vortexing solutions with Taq DNA Polymerase.
8. Seal the PCR tubes.
9. Spin the PCR tubes in order to collect the reagents at the bottom of tubes.
   Note: this spin step is essential for proper mixing of the reagents.
10. Place the PCR tubes into the real-time PCR instrument. A recommended plate layout plate layout for 22 samples, a positive control and a no-template control is shown in Table 3.
    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℃ for no more than 12 hours.

Table 3 Suggested Plate Layout (For 24 tests)

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3~9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>①</td>
<td>Sample 1</td>
<td>Sample 3</td>
<td>…</td>
<td>Sample 19</td>
<td>Sample 21</td>
<td>PC</td>
</tr>
<tr>
<td>②</td>
<td>Sample 1</td>
<td>Sample 3</td>
<td>…</td>
<td>Sample 19</td>
<td>Sample 21</td>
<td>PC</td>
</tr>
<tr>
<td>③</td>
<td>Sample 2</td>
<td>Sample 3</td>
<td>…</td>
<td>Sample 19</td>
<td>Sample 21</td>
<td>PC</td>
</tr>
<tr>
<td>①</td>
<td>Sample 2</td>
<td>Sample 4</td>
<td>…</td>
<td>Sample 20</td>
<td>Sample 22</td>
<td>NTC</td>
</tr>
<tr>
<td>②</td>
<td>Sample 2</td>
<td>Sample 4</td>
<td>…</td>
<td>Sample 20</td>
<td>Sample 22</td>
<td>NTC</td>
</tr>
<tr>
<td>③</td>
<td>Sample 2</td>
<td>Sample 4</td>
<td>…</td>
<td>Sample 20</td>
<td>Sample 22</td>
<td>NTC</td>
</tr>
</tbody>
</table>

11. Carry out real-time PCR procedure using the cycling conditions described in Table 4.
   Note:
- The reaction volume is 40 μL per well.
- Please pack the post-PCR tubes with two disposable gloves and discard properly. Do NOT open the post-PCR tubes to avoid contamination.
Table 4 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>95 °C</td>
<td>5 min</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>95 °C</td>
<td>25 s</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>64 °C</td>
<td>20 s</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>72 °C</td>
<td>20 s</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>93 °C</td>
<td>25 s</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60 °C</td>
<td>35 s</td>
<td>☆Data collection of FAM</td>
</tr>
<tr>
<td>3</td>
<td>72 °C</td>
<td>20 s</td>
<td></td>
</tr>
</tbody>
</table>

Sample Data Analysis

1. The FAM signal indicates the sample HER2 mutation status in Tubes ① and ②.
2. Ensure the calibration fluorescence is unselected, and select the sample and controls as a group, then adjust the threshold for FAM amplification curves to obtain the Ct values of the sample and controls. It is necessary to choose the reaction wells for positive control, no-template control and sample simultaneously.
3. Assess each NTC Ct value to ensure that there is no positive amplification, if the NTC has positive amplification, the data must be discarded as there may be contamination.
4. Assess each Positive Control (PC) Ct value to ensure there is positive amplification, and the Ct value should be less than 20. Otherwise, the data must be discarded.
5. Check the FAM signal from Tubes ③:
   (1) Ct value should be between 15~21 for paraffin embedded specimens, and between 13~19 for non-paraffin embedded specimens.
   (2) If the requirements of item (1) are satisfied, further analysis shall be carried out. However, if Ct value is below the corresponding range, it indicates the DNA is overloaded, the procedure should be repeated with reduced DNA.
   (3) If the external control assay fails, it shows that the DNA template contains PCR inhibitors, indicating that the DNA needs to be re-extracted. If the result in Tube ① or ② is positive, the sample is classified as positive.
6. Analysis of mutation assay results, there are 2 possible outcomes:
   (1) If the sample FAM Ct value ≥ 25, the sample is classified as negative or below the detection limit of the kit.
   (2) If the FAM Ct value < 25, the sample is classified as positive.

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 filter pipette tips to add DNA template and during the preparation of reagents.
7. To optimize the activity and performance, mixtures could always be protected from light to avoid photo bleaching.
8. Only trained professionals could use this kit. Please wear suitable lab coat and disposable gloves. The used kit should be disposed of properly.

Notes

1. Symbol for "KEEP DRY"

2. Symbol for "THIS WAY UP"

3. Symbol for "FRAGILE, HANDLE WITH CARE"

References