AmoyDx® BCR-ABL T315I Mutation Detection Kit

Detection of T315I mutation in the BCR-ABL gene

Instructions For Use

Instructions Version: B2.4
Date of Revision: October 2012

Store at -20±2 ºC
Background
Chronic Myeloid Leukemia (CML) is a clonal disease characterized by the presence of the Philadelphia (Ph+) chromosome and its oncogenic product, BCR-ABL, a constitutively active tyrosine kinase, that is present in >90% of the patients. Epidemiologic data indicates that almost 5000 new cases are reported every year and 10% of these patients eventually succumb to the disease. The treatment of CML was revolutionized by the introduction of Gleevec, a BCR-ABL tyrosine kinase inhibitor (TKI). The clinical use of specific BCR-ABL inhibitors has resulted in a significantly improved prognosis, response rate, overall survival, and patient outcome in CML patients compared to previous therapeutic regimens. However, the complete eradication of CML in patients receiving Gleevec has been limited by the emergence of resistance mostly due to mutations in the ABL kinase domain at position 315 (T315I). The second-generation BCR-ABL TKIs nilotinib (Tasigna) and dasatinib (Sprycel), showed significant activity in clinical trials in patients intolerant or resistant to Gleevec therapy, except in those patients with the T315I BCR-ABL mutation.

Intended Use
The AmoyDx® BCR-ABL T315I Mutation Detection Kit is highly selective and sensitive in the detection of T315I mutation in BCR-ABL gene. AmoyDx’s patented technology allows detection of 1% mutant DNA in a background of 99% normal DNA, while ensuring that false negatives are minimized. And this Kit is intended for research use only.

Kit Contents
This kit contains sufficient reagents to carry out 24 tests (Table 1).

<table>
<thead>
<tr>
<th>Tube</th>
<th>Reagents Supplied</th>
<th>Volume</th>
<th>Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>①</td>
<td>T315I Reaction Mix</td>
<td>1000 µL</td>
<td>FAM, HEX/VIC</td>
</tr>
<tr>
<td>②</td>
<td>BCR-ABL Taq DNA Polymerase</td>
<td>15 µL</td>
<td></td>
</tr>
<tr>
<td>③</td>
<td>BCR-ABL Mixed Standard</td>
<td>150 µL</td>
<td></td>
</tr>
</tbody>
</table>

Equipment and Reagents Not Supplied With Kit
1. Compatible PCR instruments are:
   - Stratagene Mx3000P™, Stratagene Mx3005P™, ABI7300, ABI7500.
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. The shelf-life of the kit is eight months when the kit is stored immediately upon receipt at -20±2 ℃ in a constant-temperature freezer and protected from light. Please aliquot the reagents if necessary, and store the left reagents at -20±2 ℃ immediately. The opened reagent is still valid until the expiry.

Specimen Material
Human genomic DNA is extracted from blood. DNA must be extracted from materials prior to use and stored at -20±2 ℃. Good DNA quality is essential and we recommend use of Qiagen DNA extraction kit (DNeasy Blood & Tissue kit, Cat No. 69504 or 69506, for tissue and blood specimens). The OD value of DNA samples should be measured using the spectrophotometer after extraction. The Thermo Fisher NanoDrop 1000 /2000 spectrophotometer is recommended. Make sure A260/A230 value is greater than 2.0 and 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 BCR-ABL mutations in human genomic DNA. The mutant BCR-ABL gene is amplified by the specific primers, and detected by the novel probes.

Protocol
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 BCR-ABL Mixed Standard (STD) should be analyzed during each PCR run, along with no-template controls (NTC).


2. According to the ratio of 0.25 μL BCR-ABL Taq DNA Polymerase to 35 μL BCR-ABL Reaction Mix per sample, transfer the appropriate amount of BCR-ABL Taq DNA Polymerase and BCR-ABL Reaction Mix into a sterile tube. Mix each solution for approximately 15 seconds and then centrifuge for 15 seconds.

3. Transfer 35 μL of the above master mix into PCR reaction wells.

4. Add 5 μL sample DNA (1 ~ 2 ng/μL), 5 μL BCR-ABL Mixed Standard (STD), or 5 μL ddH2O (NTC) to the appropriate PCR reaction wells. The layout for 22 samples, a positive control and a no-template control is shown in Table 2.

Table 2. Plate Layout (example for 24 tests)

<table>
<thead>
<tr>
<th>Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 3</td>
<td>Sample 4</td>
<td>Sample 5</td>
<td>Sample 6</td>
<td>Sample 7</td>
<td>Sample 8</td>
</tr>
<tr>
<td>B</td>
<td>Sample 9</td>
<td>Sample 10</td>
<td>Sample 11</td>
<td>Sample 12</td>
<td>Sample 13</td>
<td>Sample 14</td>
<td>Sample 15</td>
<td>Sample 16</td>
</tr>
<tr>
<td>C</td>
<td>Sample 17</td>
<td>Sample 18</td>
<td>Sample 19</td>
<td>Sample 20</td>
<td>Sample 21</td>
<td>Sample 22</td>
<td>STD</td>
<td>NTC</td>
</tr>
</tbody>
</table>

5. Seal the PCR tubes.

6. Spin the PCR tubes gently in a centrifuge to collect the reagents at the bottom of wells.

NOTE: This spin step is essential for proper mixing of the reagents.

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

8. Carry out real-time PCR using the cycling conditions described in Table 3.

NOTE: Make sure the total volume of solution in each well is 40 μL (35 μL reagents plus 5 μL template).

Table 3 Cycling Parameters

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>95 ℃</td>
<td>5 min</td>
<td></td>
</tr>
<tr>
<td>Stage 2</td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>95 ℃</td>
<td>25 s</td>
<td></td>
</tr>
<tr>
<td>60 ℃</td>
<td>20 s</td>
<td></td>
</tr>
<tr>
<td>72 ℃</td>
<td>20 s</td>
<td></td>
</tr>
<tr>
<td>Stage 3</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>93 ℃</td>
<td>25 s</td>
<td></td>
</tr>
<tr>
<td>56 ℃</td>
<td>35 s</td>
<td>☆ Data collection of FAM and HEX/VIC</td>
</tr>
<tr>
<td>72 ℃</td>
<td>20 s</td>
<td></td>
</tr>
</tbody>
</table>

Note: The probe settings on ABI machine: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.

Sample Data Analysis

1. The FAM signal indicates the mutation status of sample and the HEX/VIC signal indicates the internal control status. The HEX/VIC control amplifies and detects a region of genomic DNA adjacent to the BCR-ABL gene.

2. Make sure that each well gives a HEX/VIC signal. If the HEX/VIC signal gives a positive result, then continue with the analysis. If the Ct value <13, it indicates that the DNA was overloaded, and the amount of DNA should be reduced. If the HEX/VIC signal assay failed, it shows that the DNA template contains PCR inhibitors. In this case, the DNA should be re-extracted and the whole experiment should be carried out again.

3. Ensure the calibration fluorescence is unselected. Select the sample and control positions as a group. Then adjust the Threshold for FAM amplification curves to obtain the Ct values of the samples and controls.

4. The Mixed Standard FAM Ct value should be less than 23, but variation may occur due to different threshold settings on different instruments.

5. Analysis of mutation assay results.

a) Check the FAM Ct value for each sample. Based on different mutant Ct values, the detection results are divided into strong positive, weak positive or negative.

b) Negative: If the sample FAM Ct value is greater than or equal to 29 (the critical negative value), the sample is classified as negative or below the detection limit of the kit.
c) If the FAM Ct value is less than 29 (the critical negative value), please analyze the results by the following approaches:

i) **Strong Positive**: If the sample FAM Ct value is less than 26 (critical positive value), the sample is classified as strong positive.

ii) If the sample FAM Ct value is greater than or equal to 26 (critical positive value), the ∆Ct of the reaction tube shall be calculated to confirm the result. If the ∆Ct value is less than 10, the sample is confirmed as **weak positive**. If the ∆Ct value is greater than or equal to 10, the sample is classified as **negative** or below the detection limit of the kit.

d) The calculation of ∆Ct: ∆Ct = mutant FAM Ct value – internal control HEX/VIC Ct value.

The mutant Ct value indicates the Ct value of the sample mutant FAM signal; the internal control Ct value indicates the Ct value of internal control HEX/VIC signal of the sample.

**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 among different batches.
4. The kit and its contents cannot be resold or modified for resale without the written approval of AmoyDx.
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 should always be protected from light to avoid photo bleaching.
8. All the chemicals are potential hazard, only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves. The used kit should be disposed of properly.
9. AmoyDx grants the customer a non-exclusive and non-transferable license to use AmoyDx technologies.

**Notes**

1. **Symbol for "IN VITRO DIAGNOSTIC MEDICAL DEVICE".**
2. **Symbol for "KEEP DRY".**
3. **Symbol for "THIS WAY UP".**
4. **Symbol for "FRAGILE. HANDLE WITH CARE".**

**References**