AmoyDx® *BCR-ABL* Mutation Detection Kit

Instructions for Use

For Research Use Only. Not for use in diagnostic procedures.

REF 8.01.0124 24 tests/kit For Rotor-Gene 6000 (72 Wells) and Rotor-Gene Q (72 Wells)

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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* Mutation Detection Kit is a real-time PCR assay for qualitative detection of T315I mutation in *BCR-ABL* gene in human genomic DNA extracted from blood sample. This kit is intended for research use only, and intended to be used by trained professionals in a laboratory environment.

Principles of the Procedure

The kit adopts amplification refractory mutation system (ARMS) technology which comprises specific primers and fluorescent probes to detect gene mutations in real-time PCR assay. During the nucleic acid amplification, the targeted mutant DNA is matched with the bases at 3' end of the primer, amplified selectively and efficiently, then the mutant amplicon is detected by fluorescent probes labeled with FAM. While the wild-type DNA cannot be matched with specific primers, there is no amplification occurs.

The kit is composed of **T315I Reaction Mix**, ***BCR-ABL* Enzyme Mix** and ***BCR-ABL* Positive Control**.

1) The **T315I Reaction Mix** includes a mutation detection system and an internal control system. The mutation detection system is used to detect the mutation status of T315I gene (positive or negative). The internal control system is designed to detect the presence of inhibitors and monitor the accuracy of experimental operation, which may lead to false negative results.
2) The ***BCR-ABL* Enzyme Mix** contains Taq DNA polymerase for PCR amplification and uracil-N-glycosylase which is working at room temperature to prevent PCR amplicon carryover contamination.
3) The ***BCR-ABL* Positive Control** contains recombinant DNA with the T315I mutation.

Kit Contents

This kit contains the following materials:

Table 1 Kit Contents

Content	Main Ingredients	Quantity	Fluorescent signal
T315I Reaction Mix	Primers, Probes, Mg^{2+}, dNTPs	1000 µL/tube ×1	FAM, HEX/VIC
***BCR-ABL* Enzyme Mix**	Taq DNA Polymerase, Uracil-N-Glycosylase	15 µL/tube ×1	/
***BCR-ABL* Positive Control**	Plasmid DNA	150 µL/tube ×1	/

Storage and Stability

The kit requires shipment on frozen ice packs. All components 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 eight cycles.

Materials Required But Not Supplied

1) Compatible PCR instruments are: Rotor-Gene 6000 (72 Wells) or Rotor-Gene Q (72 Wells).
2) DNA Extraction Kit. We recommend use of AmoyDx® Blood DNA Kit.
3) Spectrophotometer for measuring DNA concentration.
4) Mini centrifuge with rotor for centrifuge tubes.
5) Mini centrifuge with rotor for PCR tubes.
6) Nuclease-free centrifuge tubes.
7) Nuclease-free PCR tubes and caps.
8) Adjustable pipettors and filtered pipette tips for handling DNA.
9) Tube racks.
10) Disposable powder-free gloves.
11) Sterile, nuclease-free water.
12) 1×TE buffer (pH 8.0).

Precautions and Handling Requirements

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 25 μL.
- Prior to the operation, please set up the PCR program by the following steps: ①select "**Gain Optimization**", the "**Auto Gain Optimization Setup**" window will open (see Figure 1); ②Click "**Perform Calibration Before 1st Acquisition**" and "**Optimize Acquiring**" (see Figure 2). ③Click "**OK**", then click "**Close**" to continue (see Figure 3).

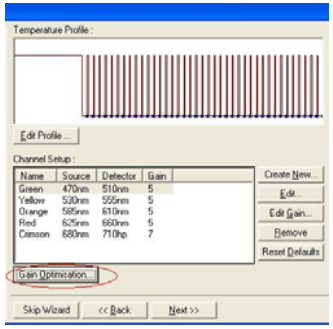

Figure 1

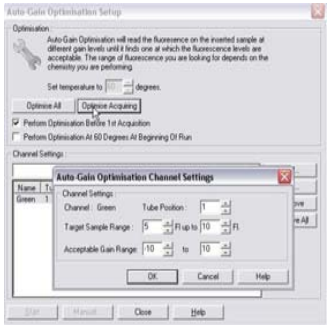

Figure 2

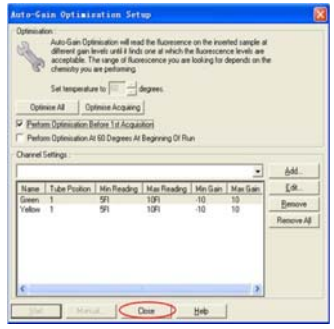

Figure 3

Assay Procedure

1. DNA Extraction

The specimen material must be human genomic DNA extracted from blood sample. DNA extraction reagents are not included in the kit. High quality DNA is essential and we recommend use of AmoyDx® Blood DNA Kit (for blood specimen). Carry out the DNA extraction according to the instructions of DNA extraction kit.

The OD_{260}/OD_{280} value of extracted DNA should be between 1.8 ~ 2.0 (measured using the spectrophotometer, the NanoDrop 1000 /2000

spectrophotometer is recommended).

The DNA concentration of extracted DNA used for PCR amplification should be 1~2 ng/μL.

Note:

- *Avoid using heparin anti-coagulated whole blood.*
- *The extracted DNA should be used immediately, if not, it should be stored at -20±5 ℃ for no more than 6 months.*
- *Before detection, dilute the extracted DNA with 1×TE buffer (pH 8.0) to designated concentration. We recommend using at least 5 μL DNA for 10 times dilution, to ensure the validity of final concentration.*

2. Mutation Detection

1) Thaw **T315I Reaction Mix** and ***BCR-ABL* 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) Briefly centrifuge ***BCR-ABL* Enzyme Mix** prior to use.
3) Prepare sufficient *BCR-ABL* Master Mix containing *BCR-ABL* Enzyme Mix and T315I Reaction Mix in a sterile tube according to the ratio in Table 2. Mix the solution thoroughly by gently pipeting it up and down more than 10 times. Centrifuge briefly.

Table 2 *BCR-ABL* Master Mix

Content	Volume per test
***BCR-ABL* Enzyme Mix**	0.25 μL
T315I Reaction Mix	22 μL
Total	**22.25 μL**

Note:

- *Every PCR run must contain one PC (Positive control) and one NTC (No template control).*
- *Do not vortex enzyme mix or any mixture with enzyme mix.*
- *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.*

4) Prepare sufficient PCR tubes for PC, NTC and samples. Dispense 22 μL *BCR-ABL* Master Mix into each of the PCR tubes.
5) Take out the sample DNA and nuclease-free water for NTC.
6) Add 3 μL nuclease-free water into NTC tube, add 3 μL each of sample DNA (1~2 ng/μL) into each sample tube, add 3 μL *BCR-ABL* Positive Control to the PC tube. Cap the PCR tubes.
7) Briefly centrifuge the PCR tubes to collect all liquid at the bottom of each PCR tube.
8) Place the PCR tubes into the real-time PCR instrument. A recommended plate layout is shown in Table 3.

Table 3 Plate Layout (example for 24 tests)

Well	1	2	3	4	5	6	7	8
A	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
B	Sample 9	Sample 10	Sample 11	Sample 12	Sample 13	Sample 14	Sample 15	Sample 16
C	Sample 17	Sample 18	Sample 19	Sample 20	Sample 21	Sample 22	PC	NTC

9) Carry out real-time PCR using the cycling conditions described in Table 4.

Table 4 Cycling Parameters

Stage	Cycles	Temperature	Time	Data collection
1	1	95℃	2.5min	/
2	15	95℃	8s	/
		64℃	10s	/
		72℃	8s	/
3	31	95℃	2s	/
		60℃	15s	Green / Yellow
		72℃	8s	/

10) Start the PCR run immediately.
11) When the PCR run is finished, analyze the data according to the "Results Interpretation" procedures.

3. Results Interpretation

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

1) For NTC: The FAM Ct value should be ≥**31**. If not, the data is *INVALID*. The sample should be retested.
2) For Positive Control: The FAM Ct value should be **< 23**. If not, the data is *INVALID*. The sample should be retested.
3) For the HEX/VIC signal for each sample, the Ct value should be >**13** and < **31**.
   a) If Ct value ≤ 13, this indicates overloading of DNA. The DNA needs to be reduced and retested. But if the FAM Ct is ≥ 29, the result will be determined as negative.
   b) If Ct value ≥ 31, this indicates the DNA degradation or the presence of PCR inhibitors. The sample should be retested with increased or re-extracted DNA. But if the FAM Ct is < 26, the result is determined as positive.

Analyze the mutation assay for each sample:

4) Check the FAM Ct value for each sample.
   a) If the mutant FAM Ct value is ≥ **29** or there is no amplification, the sample is determined as negative (no mutation detected) or under the LOD of the kit.
   b) If the mutant FAM Ct value is < **26**, the sample is determined as positive (mutation detected).
   c) If the mutant FAM Ct value is ≥ **26** and < **29**, calculate the ΔCt value to confirm the result.
      i. **ΔCt value = Mutant FAM Ct value – Internal control HEX/VIC Ct value**.
      ii. If the ΔCt value is < **10** (cut-off ΔCt value), the sample is determined as positive (mutation detected).
      iii. If the ΔCt value is ≥ **10**, the sample is determined as negative (no mutation detected) or under the LOD of the kit.

Limitations

1) The kit is to be used only by personnel specially trained with PCR techniques.
2) The kit has been validated for use with DNA extracted from human blood.
3) The kit can only detect the T315I mutation in *BCR-ABL* gene.

4) Reliable results are dependent on proper sample processing, transport, and storage.

5) The sample containing degraded DNA may affect the ability of the test to detect T315I mutation.

6) Samples with negative result (No mutation detected) may harbor T315I mutation not detected by this assay.

References

1. Jabbour E. *et al.* 2009. *Blood*. 114: 2037-43.

2. Hughes T. *et al.* 2009 *J Clin. Oncol.* 27: 4204-10.

3. Nicolini FE. *et al.* 2009. *Blood*. 114: 5271-78.

Symbols

	Manufacturer		Catalogue Number
	Batch Code		Use By
	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care
	Keep Away from Sunlight		