PAP-ARMS®

Human *BCR-ABL* Gene T315I Mutation Detection Kit

Multiplex Fluorescence Polymerase Chain Reaction

Instruction for Use

Product Name

Human *BCR-ABL* Gene T315I Mutation Detection Kit (Multiplex Fluorescence Polymerase Chain Reaction)

Packing Specification

25 Tests/Kit

Intended Use

This kit uses multiple fluorescent PCR amplification technology, and DNA extracted from peripheral blood or bone marrow as the detection sample to qualitatively detect the somatic mutation of *BCR-ABL* gene T315I in the DNA sample, provide drug scientific reference for patients with chronic myeloid leukemia (CML).

CML accounts for 15% to 20% of all leukemias, and more than 95% of CML patients have the characteristic *BCR-ABL* fusion gene. Imatinib (IM) is the first-line drug for the treatment of CML. With the progression of the disease, almost all CML blasts and 15% to 20% of CML patients with relapse after IM treatment develop resistance to IM, and the occurrence of resistance is closely related to mutations in the tyrosine kinase domain of the *BCR-ABL* fusion gene. Increasing the dose of IM or replacing second-generation tyrosine kinase inhibitors such as Nilotinib, Bosutinib, and Dasatinib is effective for most CMLs. However, CML with the *BCR-ABL* gene T315I mutation is ineffective against the above drugs. The T315I mutation is the substitution of isoleucine (Ile) at the 315th threonine (Thr) in exon 6 of the *ABL1* gene, and the base is changed from ACT to ATT. After the mutation, Ile315 cannot form hydrogen bonds with IM, and the additional carbon-hydrogen bonds on the side chain of Ile after substitution will cause steric interference, which is not conducive to the binding of IM, resulting in drug resistance. Therefore, the detection result of *BCR-ABL* gene T315I mutation is an important indicator to guide the medication of CML patients.

Technological Principles

The kit designs ARMS primers based on the sequence of designated mutation sites. The length of target sequence and internal/external control (conserved sequence of human genome) is <150bp and 100bp, respectively. For product analysis, the use of fluorescently labeled probe real-time tracking analysis makes the detection method automatic. Specific fluorescence probes are fluorescence-marked probes of specified sequences, with the reporter group at 5'-end and the quencher group at 3'-end. When the probe is complete, the fluorescence is quenched between two groups, while during the amplification of specified sequences, the reporter group will be hydrolyzed by the 5'-end exonuclease activity of Taq DNA polymerase, thus separate the two groups, and release the specific fluorescence. In other words, amplification of one DNA strand means the formation of one fluorescence molecule, thus synchronizing the accumulation of fluorescence signal with the PCR procedure. Specific kind of mutation can be detected by the kit with high specificity and high sensitivity on the real-time PCR platform. When analyzing the results, the FAM signal indicates the gene mutation and the HEX (or VIC) signal indicates internal control.

Kit Contents

The kit contains DNA polymerase, positive control and reaction reagent (Table 1), the FAM signal indicates the gene mutation and the HEX (or VIC) signal indicates internal control.

Table 1. Kit Contents

Content Name	Components	Volume	Quantity
T315I Reaction Mix	Primers, probes, Mg^{2+}, dNTPs	700 μL	2 tubes
Taq Polymerase (T315I)	Taq DNA Polymerase	10 μL	1 tube
T315I Positive Control	Positive Plasmid DNA, Wild Type DNA	35 μL	1 tube

Note: The contents of different batches cannot be mixed.

Equipment and Reagents Required

1. Commercialized nucleic acid extraction kit;

2. DNase-free and RNase-free purified water (NTC);
3. DNase-free and RNase-free pipettes, tips and PCR tubes.

Transportation, Stability and Storage

1. Storage Condition: Store the kit away from light at -20±5°C, valid for 9 months. Once opened, the kit is stable at -20±5°C until the stated expiration date. Do not use the reagents after 5 freezing-thawing cycles.
2. Transportation Condition: The kit should be transported in foam cases with ice bags, with transporting time less than one week and transporting temperature lower than 25°C.
3. Check labels for production date and expiration date of the kit.

Applicable Instruments

Stratagene Mx3000P™, ABI7500, ABI7300.

Note: For ABI instruments, define targets and passive reference as follows: Reporter Dye: FAM, VIC; Quencher Dye: TAMRA; Passive Reference: NONE.

Specimen Material

1. Recommended sample types: peripheral blood or bone marrow samples.
2. Commercialized kit is recommended to extract genomics DNA. Assess the quality of sample DNA with a microvolume UV-Vis spectrophotometer, the ratio of OD_{260}/OD_{280} should be within the range of 1.7~2.2; the concentration of sample DNA should be ≥ 2 ng/μL. Once DNA quality or quantity is not in conformity with the above requirements, re-extract DNA with new and/or larger input.
3. It is recommended to detect the extracted DNA immediately, or store it below -20°C for no more than 12 months. Do not repeated freezing and thawing for more than 5 times.

Experimental Procedure

1. Reagent Preparation(reagent reparation area)

   a). According to the number of tested samples, take the T315I Reaction Mix out of the kit. When the reagents completely thawed, vortex the tube and briefly centrifuge.

   b). Prepare sufficient T315I Master Mix according to the ratio in Table 2. If many samples will be tested, T315I Master Mix for one or two more tubes is recommended; It is recommended to perform the analysis of samples, positive controls (PC), and negative control (NTC, purified water, self-prepared) at the same time in each PCR reaction.

Table 2. T315I Master Mix

Content Name	Volume Per Test
T315I Reaction Mix	44.75 μL
Taq Polymerase (T315I)	0.25 μL
Total volume	45 μL

   c). Transfer 45 μL of each T315I Master Mix into appropriate PCR tube. Cap the tubes, then place them on the ice box for subsequent use.

2. Sample Processing (sample processing area)
   a). It is recommended to use a commercial nucleic acid extraction reagent on market to extract the sample DNA. After the extraction, the DNA concentration is measured and diluted to 2 ng/μL, which is the sample DNA to be tested.
   b). The operators remove the cap of the PCR tubes carefully, add 5 μL above sample DNA/T315I positive control/negative control to the sample tube, PC tube and NTC tube respectively. Carefully cover the cap of the strip, and move it to the amplification detection area.
3. Detection (amplification and detection area)
   a) The operators centrifugate PCR tube for 10 seconds to collect templates;
   b) The operators load the PCR tubes into the real-time PCR instrument;

c) The operators set and run the amplification program as shown in Figure 1.

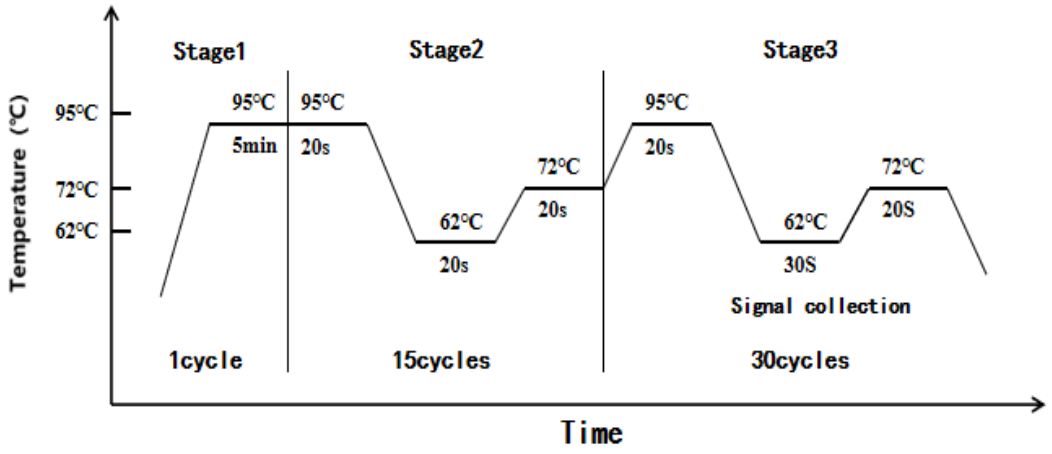

Figure 1. PCR Amplification Procedure

d). The operators perform real-time PCR and save the file

e). The operators handle the stripes properly after experiment; do not remove the caps in case of contamination.

Positive Judgment Value

1. Sample Ct value: use the amplification curve Ct value calculated by the instrument software or determine the amplification Ct value at the inflection point where the amplification curve rises according to the actual situation.
2. Result Judgment: If the Ct value of the sample is less than 28, a positive call is returned; if the amplification curve does not rise, a negative call is returned (or lower than the detection limit of the kit); if the Ct value of the sample is $28 \leq Ct < 30$, increase the amount of sample and retest. If the retest result Ct value is $28 \leq Ct < 30$, it is judged as negative (or lower than the detection limit of the kit); if the retest result Ct value is less than 28, it is judged as positive.

Interpretation of Results

1. NTC: there should be no amplification curves of FAM in NTC reaction tube, or else, call the result invalid. Occasionally, amplification curve of HEX (or VIC) generates in NTC tube, which has no influence on result interpretation.
2. PC: the FAM Ct of PC is always less than 24, HEX Ct of PC is always less than 18, but varies among different instruments due to various fluorescence thresholds.
3. Internal control: The HEX (or VIC) Ct of every sample reaction tube should be 12 ~ 20, which must be qualified before proceeding to further analysis; if the HEX (or VIC) Ct is less than 12, that indicates excessive DNA amount, dilute sample DNA for a new detection; if the HEX (or VIC) Ct is greater than 20, that indicates insufficient DNA amount or that sample DNA was contaminated by PCR inhibitor, in this case, re-extract sample DNA for a new detection.

Limitation of the Kit

1. Negative results could not exclude the existence of *BCR-ABL* gene T315I mutation; cases like inadequate tumor cells, DNA degradation or insufficient DNA amount may lead to negative results as well.
2. Situations that may result in false negative or false positive result include but not limit to unreasonable sample collection, transportation, improper experimental operations or environment.
3. The kit is only intended for the qualitative detection of specific T315I mutations of human *BCR-ABL* gene.
4. The kit is only applicable with the stated sample types and detection system, including specified instruments, DNA extraction kit and analytical assay.

Physical Performance

1. The kit should be of neat appearance, clear labels, and of no leakage. When unfrozen, the reagents shall be clear, without sediments.
2. This kit can detect the T315I mutation in 10ng DNA samples as low as 1%.
3. The consistency rates of positive control are 100%.
4. The consistency rates of negative control are 100%.
5. The coefficient of variation (CV %) of 10 Ct values by detecting designated sample for 10 repetitive times should be less than 10%.
6. There's no nonspecific product with up to 200 ng wild-type DNA sample.

Precautions and Warning

1. Please read the IFU carefully in prior to the use of the kit.
2. Avoid repetitive freezing and thawing reagents.
3. Perform quality control of DNA after extraction; proceed to sample detection immediately or store sample DNA properly.
4. Do not substitute any content of the kit; do not mix contents of different batches.
5. Pay special attention to the use of positive control to prevent contamination of reagents or resulting in false positive results.
6. Be cautious of contamination from external DNA; when sampling, always pipette NTC and sample DNAs before positive control; segregate areas for reagent preparation and sample processing; use dedicated pipettes and tips for reagent preparation and template addition, respectively.
7. Sterilize the environment and pipettes with 10% hypochlorous acid, or 75% ethyl alcohol, or UV radiation.
8. All the reagents in use have potential hazard. Only people who have work permit for PCR laboratories are allowed to use this kit. It is suggested to wear proper protective suit and gloves. For first-use of this kit, you may receive training by our technical supports.
9. All samples including positive control in the kit should be considered potential infectious substances. They should be handled carefully.

Notes

Symbol	Legend
	Indicates the need for the user to consult the instructions for use.
	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device.
	Indicates the date when the medical device was manufactured.
	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	Indicates the temperature limits to which the medical device can be safely exposed.
	Indicates the date after which the medical device is not to be used.
	This is the correct upright position of the distribution packages for transport or storage.
	Indicates a medical device that needs to be protected from moisture.
	Indicates a medical device that needs protection from light sources.

	Indicates the medical device manufacturer.
	Indicates the authorized representative in the European Community/European Union.
	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC.

Literature references

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