BCR-ABL1 Kinase Domain Mutation Screening Kit

LENA BCR-ABL1
BCR-ABL1 KINASE DOMAIN MUTATION SCREENING KIT

CE
IVD

This package insert must be read carefully prior to use and should be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

REF **803417**

20 Tests per Kit
***In Vitro* Diagnostic Medical Device**
For Professional Use Only

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1. INTENDED USE

BCR-ABL1 Kinase Domain Mutation Screening Kit is an *in vitro* diagnostic reagent used for qualitative detection of mutations. The kit employs PCR and melting curve analysis methods, and covers the detection of 40 resistance mutations from exon 4 to exon 9 of ABL1 gene.

2. PRINCIPLE OF THE PROCEDURES

BCR-ABL1 Kinase Domain Mutation Screening Kit employs dually labeled probes to target amplicons generated by PCR; then uses melting curve analysis to generate the minus derivative of the fluorescence intensity versus temperature, and compares the melting temperature (T_m) to gain mutation information of the sequences.

3. REAGENTS AND MATERIALS SUPPLIED

BCR-ABL1 Kinase Domain Mutation Screening Kit contains reagents for 20 tests, components are tabulated as below.

Component	Volume (μL)	Description
RT Mix	297	For reverse transcription reaction
RT Enzyme	36	
Preamplification Mix	180	For preamplification reactions
Preamplification Enzyme	10	
KM PCR Mix A~D	436	For qPCR reaction
LF Polymerase	45	
KM Positive control	120	For positive control
KM Negative control	120	For negative control
Mineral oil	2400	/
KM Diluent	2400	/
User manual	1 piece	/

Note: Do not interchange reagents from different production lots.

4. INSTRUMENT

This kit is suitable for real-time PCR cyclers with FAM, HEX, ROX and Cy5 detection channels with melting curve analysis function.

5. MATERIALS REQUIRED BUT NOT PROVIDED

- Thermal Heating Block
- Disposable powder-free gloves
- Adjustable pipettes and sterile filtered pipette tips, 1.5 mL and 2.0mL microcentrifuge tubes
- Vortex mixer
- High speed centrifuge for 1.5 mL and 2.0mL microcentrifuge tubes
- Hand-held centrifuge for 0.2 mL optical PCR tubes, 8-tube strips or 96 plates as well as 1.5 mL microcentrifuge tubes
- 0.2 mL optical PCR tubes, 8-tubes strips with Optical Caps or 96 plates with sealing film which match the SLAN 96s/96p/48p.

6. STORAGE CONDITIONS AND SHELF-LIFE

The kit should be stored at -25~-18℃ away from light for up to 6 months. Repeated freezing and thawing are preferably not more than 3 times. The kit requires cold-chain-transportation. Production date and expiration date are shown on the package label.

7. SAMPLE REQUIREMENTS

Collection of primary samples

Collect 2~3 mL bone marrow or whole blood sample according to routine collection procedure using EDTA or sodium citrate for anticoagulation. DO NOT use heparin due to its inhibition to PCR reaction. Total RNA should be isolated immediately after collection or should be stored at 2-8 ℃ for no more than 24 hours as RNA degrades easily. If not, remove the red blood cells, then add 1 mL of trizol per 5~10×106 leukocytes and store at -70 ℃ for no more than one month.

Extraction of total RNA

Isolate total RNA from bone marrow or whole blood by an appropriate method, use of trizol is recommended. Since the quality of RNA would affect the test results, it is suggested that the concentration and purity of RNA should be measured with UV spectrophotometer. The ratio of A260/A280 should be within 1.9-2.1, and the ratio of A260/A230 should be above 2.0. In order to avoid RNA degradation, the isolation should be carried out in RNase-free environment, such as a biological safety cabinet or clean bench. Make sure all microcentrifuge tubes, PCR tubes and other consumables are free of RNase. Wear latex gloves, masks or use other protective methods throughout the process to avoid RNase contamination.

Mass concentration of total RNA used for RT

After isolation, reverse transcription must be done immediately, or the RNA should be stored at -70 °C for no more than 3 months. For accurate detection, the recommended mass concentration of total RNA should be between 20 ng/μL and 200 ng/μL. Before reverse transcription, it is suggested that the integrity of RNA should be evaluated if the laboratory conditions permit. Agarose or denaturing agarose gel electrophoresis is recommended to assess RNA integrity. A 28s rRNA/18s rRNA ratio of 2 means good integrity.

8. TEST PROCEDURE

8.1 Reverse Transcription (RT)

a. Thaw the RT Mix at room temperature (15~25 °C) and vortex it for 10 seconds. RT Enzyme is no need to be vortexed. Then spin them briefly to collect the reagents at the bottom of the tubes.

b. Prepare RT reaction mix using PCR tubes for 20 μL reaction volume as shown in the table below.

Reagents	Volume (μL)/reaction
RT Mix	13.5
RT Enzyme	1.5
Total RNA	5

One RT reaction is for one sample use only. RT reaction mixes for multiple samples should be pre-mixed as a master mix with 5 % overage to cover pipetting losses. Spin the PCR tubes of RT reaction mix briefly. The whole preparation process should be completed within 2 hours and the prepared RT reaction mix should be used for the next step within 1 hour.

c. Place the PCR tubes of RT reaction mix in Thermal Cycler, incubate at 37 °C for 15 minutes for cDNA synthesis, then 85 °C for 5 seconds for RT enzyme inactivation.

8.2 Preamplification reactions

a. First, take the preamplification reaction solution and mineral oil out of the package, and place them on a test tube rack to equilibrate to room temperature, then shake and mix for 20 seconds, and centrifuge at 3000 rpm for 5 seconds to collect the tube wall liquid to the bottom of the tube.

b. Prepare the preamplification reaction solution as the following table into a 1.5 mL centrifuge tube, and then subject to vortex for several seconds followed by a brief spin. The prepared PCR reaction solution must be stored at -18°C or below and used within 4 hours.

Component	Volume
Preamplification Mix	(n+1) × 7.8 μL
Preamplification Enzyme	(n+1) × 0.2μL
Total volume	8.0 μL / reaction

Note: n = number of reactions (cDNA specimens plus controls). Prepare enough components for one extra specimen (n+1) to allow for sufficient overage for the PCR setup.

c. The preamplification reaction solution was distributed into 0.2 mL PCR reaction tubes respectively, 8 μL for each tube, and add 20 μl mineral oil to each tube.

d. Add 2 μL of cDNA to each PCR tube and cap the tube immediately. Centrifuge the PCR tube at 3000 rpm for a few seconds, and collect the liquid remaining on the tube wall to the bottom of the tube.

e. Place the pre-amplification mixture on a common PCR machine and perform the pre-amplification reaction according to the following procedure.

Stage		Condition	Cycle number
Preamplification Reaction Program	Initial Denaturation	95°C, 5 minutes	1
	PCR Cycling Program	95°C, 40 seconds 61°C, 1 minute 72°C, 2 minutes	20
	PCR re-extension	72°C, 7 minutes	1
		4°C, 1 minute	1

8.3 PCR and melting curve analysis

a. Add 90 μL of KM diluent to the pre-amplified product for dilution, cover the tube tightly, turn it upside down, mix gently for 20 seconds, and centrifuge at 3000 rpm for 5 seconds to collect the tube wall liquid to the bottom of the tube for use.

b. Take KM PCR Mix A~D out of the package and place it on a test tube rack to equilibrate to room temperature, then shake and mix for 20 seconds, and centrifuge at 3000 rpm for 5 seconds to collect the liquid from the tube wall to the bottom of the tube.

Component	Volume
KM PCR Mix A/B/C/D	(n+1) × 19.6 μL
LF Polymerase	(n+1) × 0.4μL
Total volume	20.0 μL / reaction

Note: n = number of reactions (DNA specimens plus controls). Prepare enough components for one extra specimen (n+1) to allow for sufficient overage for the PCR setup.

c. The PCR reaction solution was distributed into 0.2 mL PCR reaction tubes respectively, 20 μL for each tube. Add 5 μL of the corresponding diluted pre-amplification product, KM positive control and KM negative control to each PCR tube, and close the tube cap immediately. After adding the sample, centrifuge the PCR thin-walled reaction tube at 3000 rpm for a few seconds, so that the sample remaining on the tube wall enters the bottom of the tube.

Note: The pipette tip needs to be extended under the mineral oil surface to aspirate the pre-amplified products.

d. Place PCR tubes, strips of 8 or 96 plates into the thermal cycler and run the preset program below.

Stage		Condition	Cycle number
PCR Reaction Program	UNG Treatment	50°C, 2 minutes	1
	Initial Denaturation	95°C, 2 minutes	1
	PCR Cycling Program	95°C, 15 seconds 60°C, 35 seconds collect the fluorescent signal in FAM, HEX, ROX and Cy5	50
	PCR re-extension	35°C,30 minutes	1
Melting analysis program		95°C, 2 minutes 45°C, 2 minutes 45°C ~ 95°C, collect the fluorescent signal at FAM, HEX, ROX and Cy5 channels; temperature increasing rate: 0.04°C/s. 50°C, 30 seconds	1

e. After the program finished, the thin-walled PCR reaction tubes (closed) should be put into a self-sealing (zip) bag and sealed tightly (treated as pollutant source).

9. REFERENCE VALUE

The controls in the kit must meet the following requirements, otherwise the experiment will be considered invalid. The T_m value range of positive control in each channel of A、B、C and D reaction is shown as follows:

Reaction system A, FAM channel: 82.39°C±1.0°C; HEX channel: 70.22°C±1.0°C.

Reaction system B, HEX channel: 65.41°C±1.0°C; ROX channel: 67.46°C±1.0°C.

Reaction system C, FAM channel: 82.34°C±1.0°C; HEX channel: 68.04°C±1.0°C.

Reaction system D, HEX channel: 67.73°C±1.0°C; CY5 channel: 70.67°C±1.0°C.

The T_m values listed above were obtained from one particular SLAN-96 instrument, and may change slightly when using other instruments. Always calibrate the above T_m values with the positive control in each run.

The T_m values will be given automatically by the instrument. When more than one T_m value is obtained by the instrument, please refer to the positive and the negative controls to select a valid T_m value. Obtain T_m values manually, when the instrument could not report T_m values automatically.

10. EXPLANATION OF RESULT

1. **Interpretation of positive control**: For each run, the T_m value of positive control of all channels should be within the range of reference values, otherwise the experiment will be considered invalid.
2. **Interpretation of negative control:** There should be no signal observed for negative control and this indicates that there is no contamination during RNA extraction or detection.
3. **Interpretation of specimens：**

Detection channel	Type of mutation		T_m value
	Nucleic acid variation	Protein variation	
A-FAM	c.1135G>A	V379I	62.15±1.0°C
	c.1076T>G	F359C	70.20±1.0°C
	c.944C>T	T315I	82.39±1.0°C
A-HEX	-	-	70.22±1.0°C
A-ROX	c.730A>G	M244V	57.40±1.0°C
	c.1187A>G	H396R	61.68±1.0°C
	c.827A>G	D276G	65.86±1.0°C
	c.1052T>C	M351T	70.01±1.0°C
A-Cy5	c.749G>A	G250E	64.78±1.0°C
	c.1159T>A	L387M	70.22±1.0°C
	c.951C>G	F317L	80.11±1.0°C
B-FAM	c.1187A>C	H396P	68.28±1.0°C
	c.1150C>A	L384M	82.29±1.0°C
B-HEX	-	-	65.41±1.0°C
B-ROX	c.892C>G	L298V	57.18±1.0°C
	c.1064A>C	E355A	61.78±1.0°C
	c.931T>A	F311I	67.46±1.0°C
	c.1064A>G	E355G	71.70±1.0°C
	c.756G>C	Q252H	85.66±1.0°C
B-Cy5	c.743T>G	L248R	64.59±1.0°C
	c.756G>T	Q252H	69.12±1.0°C
C-FAM	c.895G>C	V299L	64.03±1.0°C
	c.949T>A	F317I	69.53±1.0°C
	c.758A>T	Y253F	77.91±1.0°C

	c.1349A>G	E450G	82.34±1.0℃
C-HEX	-	-	68.04±1.0℃
C-ROX	c.951C>A	F317L	57.09±1.0℃
	c.1375G>A	E459K	67.56±1.0℃
	c.949T>C	F317L	71.60±1.0℃
	c.1250C>A	S417Y	86.03±1.0℃
C-Cy5	c.757T>C	Y253H	65.90±1.0℃
	c.895G>T	V299L	70.65±1.0℃
	c.949T>G	F317V	80.20±1.0℃
D-FAM	c.950T>G	F317C	62.01±1.0℃
	c.1075T>A	F359I	70.29±1.0℃
	c.943A>G	T315A	82.47±1.0℃
D-HEX	-	-	67.73±1.0℃
D-ROX	c.1075T>G	F359V	56.84±1.0℃
	c.1057T>C	Y353H	61.47±1.0℃
	c.1376A>G	E459G	65.76±1.0℃
	c.763G>A	E255K	71.63±1.0℃
D-Cy5	c.764A>T	E255V	65.15±1.0℃
	c.1457T>C	F486S	70.67±1.0℃
	c.742C>G	L248V	81.45±1.0℃

Note: Detection channels A-HEX, B-HEX, C-HEX, D-HEX are the internal reference gene ABL1.

Warning: Environmental pollution in laboratory, reagent contamination, and cross contamination of specimens will lead to false positive; improper transportation and/or storage, incorrect reagent preparation may lower the accuracy of the test and lead to a false negative or inaccurate detection results.

11. LIMITATION OF THE METHOD

1) The kit can only detect 40 resistance mutations from exon 4 to exon 9 of ABL1 gene, other BCR-ABL1 kinase domain mutations are not covered.
2) The Rm values cannot be used for quantification of the mutation level.
3) The low level of fusion transcripts in samples can result in false negative results because of the kit's limit of detection.

12. PERFORMANCE CHARACTERISTICS

The limit of detection: 1000 copies per reaction. The detectable mutation rate of 40 mutations in the wild-type background was 5% to 10%.

13. WARNING AND PRECAUTIONS

1) The kit is for *in vitro* diagnostic use, and should be performed by trained and validated laboratory personnel. The instruction must be followed exactly to get accurate results.
2) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and specimens are handled.
3) The test results should not be used as the only reference for treatment planning. Clinicians should make comprehensive decision in consideration of patients condition, drug indications, therapeutic response and so on.
4) Please strictly follow the management standard of gene testing laboratory: such as operation in special areas; no cross use of gloves, pipettes, etc. to avoid cross-contamination; the operation area should be sterilized and disinfected with 1% sodium hypochlorite, 75% ethanol, 1 mol/L hydrochloric acid or UV light at regular intervals.
5) All materials used, including reagents and specimens, should be disposed in a manner that will inactivate infectious

agents. Each reagent in the kit needs a brief centrifugation after fully thawing and mixing. Air bubbles should be avoided during the dispensing of reaction mixture. Ensure that the reaction tubes are all tightly closed to avoid instrument contamination.

6) Each trial should contain a positive control and a negative control.
7) All reagents should be protected from light.
8) Repeated freezing and thawing of the control reagents is preferably not more than 3 times. If repeated use is required, please aliquot reagents into appropriate amount after the first thawing and store all aliquots at -18°C or below.
9) Do not interchange reagents from different kits unless the lots are identical and do not use the reagents beyond their expiry date.
10) After the reaction, remove the PCR tubes (closed) and put them into a self-sealing (zip) bag and seal tightly, treat as a biohazardous source.

Key to symbols used in the package:

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

 Manufacturer

 Production Date

CE Mark

 Store between -25 °C and -18 °C

 Expiration Date

 Do Not Reuse

 CAUTION

 Consult Instructions for Use

 Number of Tests

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Ver. A/1

LENA BCR-ABL1
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