BCR-ABL1 p210 Kit

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 **803318**

20 Tests per Kit
***In Vitro* Diagnostic Medical Device For Professional Use Only**
FOR RESEARCH USE ONLY – NOT FOR USE IN DIAGNOSTIC PROCEDURES

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1. INTENDED USE

BCR-ABL1 P210 Kit is intended to quantify the BCR-ABL1 P210 transcripts in human bone marrow or whole blood samples of leukemia patients. The results obtained from the kit provides information about efficiency of treatment or Minimal Residual Disease (MRD) in patients.

2. PRINCIPLE OF THE PROCEDURES

BCR-ABL1 P210 Kit is a RT-qPCR based assay for quantification of BCR-ABL1 p210 transcripts. The kit contains RT reaction mix and qPCR mix. cDNA is synthesized by adding purified total RNA to the RT reaction mix. The resulting cDNA is added to 2 PCR reaction tubes, which contain specific PCR primers and probes for BCR-ABL1 p210 and ABL1. The qPCR is performed in a real-time thermal cycler with optical filters for FAM dye. After PCR, a linear regression curve is calculated from the standards for each gene and used for calculating the copy number of each gene in an unknown sample. BCR-ABL1 p210 transcripts levels will be expressed as percentage of BCR-ABL1 p210 over ABL1.

3. REAGENTS AND MATERIALS SUPPLIED

BCR-ABL1 P210 Kit contains reagents for 20 tests, components are tabulated as **Table 1**.

Table 1. Reagents and Materials in the Kit

Component	Volume (μL)	Description
RT Mix	297	For reverse transcription reaction
RT Enzyme	36	
B/A 210 PCR Mix	436	For qPCR reaction
ABL1 PCR Mix	436	
LF Polymerase	15	
B/A 210 Standards 4 dilutions (1×10^3, 1×10^4, 1×10^5, 1×10^6 copies/μL)	40	For plotting of linear regression curve
ABL1 Standards 4 dilutions (1×10^3, 1×10^4, 1×10^5, 1×10^6 copies/μL)	40	
dd H_2O	100	For negative control
User manual	1 piece	/

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Real-time thermal cycler with FAM channel. Such as Bio-Rad CFX96, Mx3005P/3000P, ABI7500, Light Cycler 96 and SLAN 96s/96p/48p
- Thermal Heating Block
- Disposable powder-free gloves
- dd H_2O
- Adjustable pipettes and sterile filtered pipette tips
- Vortex mixer
- Desktop centrifuge for 0.1 ml or 0.2 ml optical PCR tubes, 8-tubes strips or 96 plates as well as 1.5 ml microcentrifuge tubes
- 1.5 ml microcentrifuge tubes
- 0.1 ml or 0.2 ml optical PCR tubes, 8-tubes strips with optical caps or 96 plates with sealing film which match the Bio-Rad CFX96, Mx3005P/3000P, ABI7500, Light Cycler 96 or SLAN 96s/96p/48p

5. STORAGE CONDITIONS AND SHELF-LIFE

THE kit with all components is stable for 12 months when stored between -25 °C and -18 °C. B/A 210 PCR Mix and ABL1 PCR Mix must be protected from light to avoid bleaching of the probes. The expiration date of each component is printed on each tube label. Frequent freeze-thaw cycles (>3x) of the kit should be avoided. The kit should be shipped at low temperature.

6. SAMPLE REQUIREMENTS

COLLECTION OF PRIMARY SAMPLE

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 5~10×10^6 leukocytes and store at -70 ℃ for no more than one month.

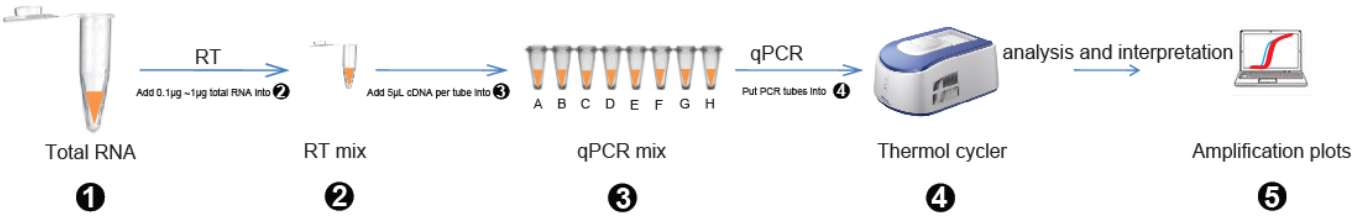
EXTRACTION OF TOTAL RNA

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.

7. TEST PROCEDURE



❶ Add 5 μL (0.1 μg~1 μg) total RNA into RT reaction mix with a total volume of 20 μL

❷ cDNA is synthesized

❸ Add 5 μL of cDNA and BCR-ABL1 p210 standards into each PCR tubes with B/A 210 PCR Mix, add 5 μL of cDNA and ABL1 standards into each PCR tubes with ABL1 PCR Mix

❹ Put PCR tubes with PCR mixes into thermal cycler and start the qPCR program

❺ Amplification plots are analyzed

Figure 1. Test procedure at a glance.

7.1 REVERSE TRANSCRIPTION (RT)

a. **THAW** the RT Mix at room temperature (15~25 ℃) 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 **Table 2**:

Table 2. RT Reaction Mix Preparation

Reagents	Volume for one Reaction (μL)
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 ℃ for 15 minutes, then 85 ℃ for 5 seconds.

d. **VORTEX** for 10 seconds and spin the PCR tubes briefly for downstream testing.

7.2 Q-PCR

a. **THAW** BCR-ABL1 p210 PCR Mix, ABL1 PCR Mix, Standards and dd H_2O to room temperature (15~25 ℃). Vortex BCR-ABL1 p210 PCR Mix and ABL1 PCR Mix for 10 seconds, and Standards for 30 seconds. LF polymerase and dd H_2O is no need to be vortexed, then spin all these tubes briefly.

b. **PREPARE** master PCR mixes using 1.5 mL microcentrifuge tubes for 20μL reaction volume as **Table 3**.

Table 3. Master PCR Mix Preparation

Reaction	Reagents	Volume (μL)
B/A 210 PCR Mix	B/A 210 PCR Mix	19.8n*
	LF Polymerase	0.2n
ABL1 PCR Mix	ABL1 PCR Mix	19.8n
	LF Polymerase	0.2n

*n equals the number of samples plus 6, for example, if there are x samples need to be tested in this run, n (n=x+6, the "6" is for standards, dd H_2O and pipetting errors respectively) tubes of PCR mix should be prepared.

c. **VORTEXING** the master PCR mix for 10 seconds and spin briefly.

d. **DISPENSE** 20 μL of each master PCR mix per well into optical PCR tubes, 8-tubes strips or 96 plates.

e. **ADD** 5 μL of each cDNA templates, standards, dd H_2O into PCR mix.

f. **SPIN** the PCR tubes briefly to collect the contents at the bottom of the tubes.

g. **PLACE** the PCR tubes, 8-tubes strips or 96 plates in the thermal cycler and run the pre-set program (see **Table 4**):

Table 4. Program of qPCR

Stage	Condition	Cycle number
UNG pre-treatment	50 ℃, 2 minutes	1
Initial denaturation	95 ℃, 10 minutes	1
PCR cycling program	95 ℃, 30 seconds 60 ℃, 1 minute, collect the fluorescent signal in FAM	50

h. **After** the program is finished, put the PCR tubes (closed) into a self-sealing (zip) bag, sealed tightly, and treat as

pollutant source.

8. ANALYSIS AND INTERPRETATION

8.1 Cq determination

After the run of qPCR, a threshold line should be carefully set up to allow accurate Cq (shown in **Figure 2**). Note: Threshold line setting should automatically output by the instrument. If the signal occurs too early or fluctuates significantly, the automatic threshold line may be not suitable for data processing. Manually set up baseline threshold according to the original amplification curve and the instruction of thermal cycler instead.

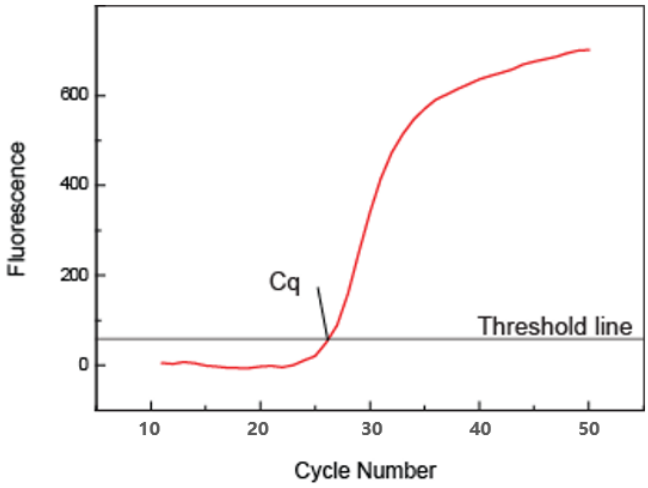


Figure 2. Cq is the intersection between the amplification curve and the threshold line.

8.2 dd H_2O

No signal should be detected for the dd H_2O in FAM channel. If not, the run is invalid and should be repeated.

8.3 Standards

The Cq values of the Standards in BCR-ABL1 p210 reaction and ABL1 reaction should be less than 40 cycles. The correlation coefficient of the standard curve is between 0.99~1.0, and the slope is between -3.0~-4.0. If not, the run is invalid and should be repeated.

8.4 Samples

a. The Cq values in ABL1 reaction should be less than 40.

b. Cq values for positive sample signals should be below 40. Cq values above 40 might be non-specific amplification (shown in **Figure 3**). Repeat the test with fresh RNA, if the second test is positive, the sample is positive for the corresponding fusion gene. Other methods are recommended to be used to confirm the results from positive tests with Ct values above 40.

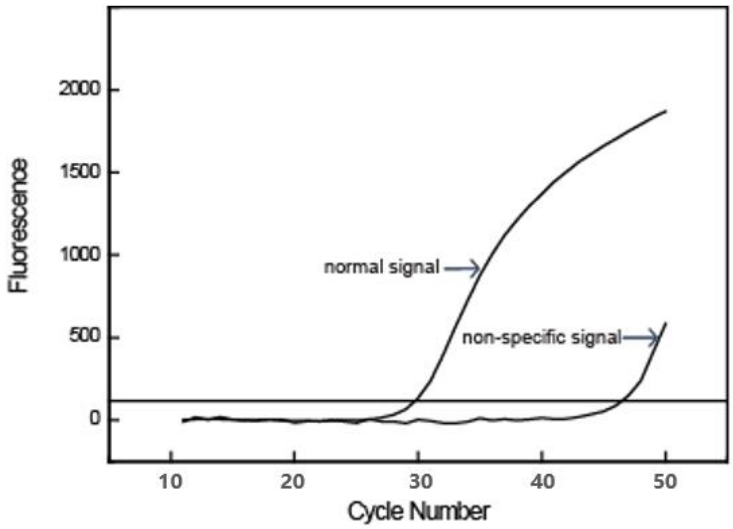


Figure 3. Non-specific signal in sample

c. Read Cq values of BCR-ABL1 p210 and ABL1 of the sample. The gene copy number was determined by standard curve, and the results were calculated according to the formula:

Quantitative value (%) = BCR-ABL1 p210 copy number/ ABL1 copy number ×100%

9. LIMITATION OF THE METHOD

THE kit can only quantify samples with gene copy number between 10^3 to10^6 copies, if not, the sample should be diluted or added more to satisfy the requirement.

10. PERFORMANCE CHARACTERISTICS

LIMIT OF DETECTION: 100 copies/reaction.

11. WARNING AND PRECAUTIONS

THE kit is for *in vitro* diagnostic use and should be performed by professionals. The instruction must be followed exactly to get accurate results.

- Do not pool reagents from different kits or lots, do not use after the expiration date printed on the external box.
- Use aerosol barrier pipette tips.
- Laboratory workbenches, pipettes and other consumables must be cleaned with bleach regularly.
- Opening PCR tubes after amplification should be avoided in order to minimize the risk of contamination.
- Gloves should be worn during the whole operation.
- The test results should not be used as the only reference for treatment planning. Clinicians should make comprehensive decision in consideration of patient condition, drug indications, therapeutic response and so on.

Key to symbols used in the package:

REF Catalogue Number

IVD In Vitro Diagnostic Medical Device

Manufacturer

Production Date

CE Mark

-18°C / -25°C Store between -25 °C and -18 °C

Expiration Date

Do Not Reuse

CAUTION

Consult Instructions for Use

Number of Tests

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LENA BCR-ABL1 P210 KIT

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E-mail: peter@lotusnl.com

Ver. A/1