LENA PML-RARα Genotyping 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 **803216**

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

Table of Contents

1. INTENDED USE ... 2
2. PRINCIPLE OF THE PROCEDURES ... 2
3. REAGENTS AND MATERIALS SUPPLIED ... 2
4. MATERIALS REQUIRED BUT NOT PROVIDED ... 2
5. STORAGE CONDITIONS AND SHELF-LIFE ... 2
6. SAMPLE REQUIREMENTS ... 3
7. TEST PROCEDURE ... 3
8. ANALYSIS AND INTERPRETATION ... 5
9. LIMITATION OF THE METHOD ... 6
10. PERFORMANCE CHARACTERISTICS ... 6
11. WARNING AND PRECAUTIONS ... 6

1. INTENDED USE

PML-RARα Genotyping Kit is a qualitative *in vitro* diagnostic test for differentiating of PML-RARα L, PML-RARα S and PML-RARα V three splice variants with leukemia. The kit detects RNA transcripts of fusion genes extracted from human bone marrow or whole blood samples with PML-RARα. The results obtained from the kit provides more information of PML-RARα to professionals for comprehensive consideration associated with patient condition, drug indication, therapeutic response and other detection findings.

2. PRINCIPLE OF THE PROCEDURES

PML-RARα Genotyping Kit is a RT-qPCR based assay for differentiating the subtype of L, S and V. Included in the kit are 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 qPCR reaction tube, which contain specific PCR primers and probes for detection of fusion genes and an internal control gene of GUSB. The qPCR is performed in a real-time thermal cycler with optical filters for the detection of FAM, HEX, ROX and Cy5 fluorescence signals. Amplification plots and the resulting Cq (Quantification cycle) values are used for the identification of the fusion gene subtype. Detection of the GUSB gene is an internal control for the integrity of the RNA sample and functionality of both cDNA and qPCR reactions.

3. REAGENTS AND MATERIALS SUPPLIED

PML-RARα Genotyping Kit contains reagents for 20 tests, components are tabulated as below (table 1):

Table 1. Reagents and Materials in the Kit

Component	Volume (μL)	Description
RT Mix	297	For reverse transcription reaction
RT Enzyme	36	
P/R PCR Mix	436	For qPCR reaction
LF Polymerase	10	
P/R L Positive Control	40	For positive control
P/R S Positive Control	40	
P/R V Positive Control	40	
dd H_2O	200	For negative control
User manual	1 piece	/

4. MATERIALS REQUIRED BUT NOT PROVIDED

- Real-time thermal cycler with FAM, HEX, ROX and Cy5 detection channels. 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-tube Strips or 96 plates as well as 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. The qPCR reagents including B/A 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 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 °C for no more than 24 hours as RNA degrades easily. If not, remove the red blood cells, then add 1 mL of trizol per $5\sim10\times10^6$ leukocytes and store at -70 °C for no more than one month.

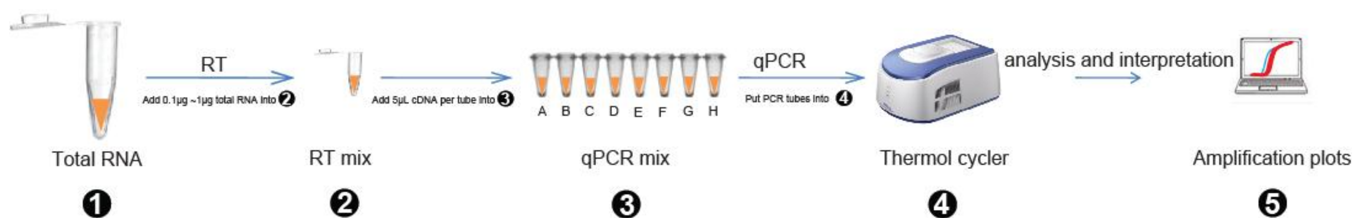
Extraction of total RNA

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. If not, the Cq values of Cy5 from P/R PCR Mix-1 for each total RNA will be above 25 cycles.

7. TEST PROCEDURE



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

❷ Synthesis of cDNA

❸ Add 5 μL of cDNA, dd H_2O, P/R L Positive control, P/R S Positive control, P/R V Positive control into each PCR tube

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

❺ Analyze amplification plots.

Figure 1. Test procedure at a glance.

7.1 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 Table 2:

Table 2. RT Reaction Mix Preparation

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

One RT reaction is for one sample use only. RT reaction mixes for multiple samples (as well as Positive Control) should be pre-mixed as a master mix with 5 % overage to cover pipetting losses. Spin the PCR tubes of RT reaction mix for 5 seconds at 3000 rpm/min. 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, then 85 °C for 5 seconds.

7.2 Q-PCR

a. **THAW** all the P/R PCR Mix-1, Positive Control and ddH_2O to room temperature (15~25 °C). Vortex P/R PCR Mix-1 for 10 seconds, and Positive Control for 30 seconds. LF polymerase and ddH_2O is no need to be vortexed, then spin all these tubes briefly.

b. **PREPARE** master PCR mix using 1.5 mL microcentrifuge tubes for 20 μL reaction volume as follows (**Table 3**):

Table 3. Master PCR Mix Preparation

Reaction	Reagents	Volume (μL)
PCR Mix	P/R PCR Mix-1	19.8n*
	LF Polymerase	0.2n

*n equals the number of samples plus 5, for example, if there are x samples need to be tested in this run, n (n=x+5, the "5" is for P/R L Positive Control, P/R S Positive Control, P/R V Positive Control, ddH_2O and pipetting errors respectively) tubes of PCR mix should be prepared.

c. **VORTEXING** the master PCR mix-1 for 10 seconds and spin the tubes briefly.

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

e. **ADD** 5 μL of each cDNA templates and positive control as well as ddH_2O into PCR mix. A Layout example is shown in **Figure 2** (take 8-tubes strips for example).

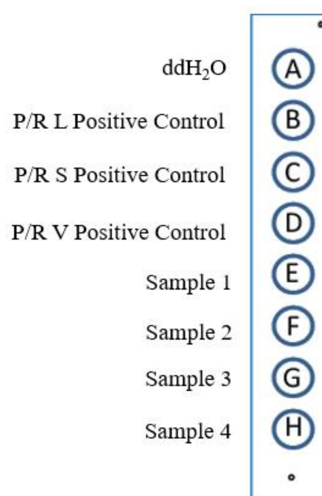


Figure 2. Sample and control distribution in the 8-tubes strips.

f. **SPIN** the PCR tubes for 5 seconds briefly.

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 °C,2 minutes	1
Initial denaturation	95 °C,10 minutes	1
Touchdown cycling program	95 °C,20 seconds 65 °C,1 minute (decreased 1 °C per cycle) 72 °C,1 minute	10
PCR cycling program	95 °C,20 seconds 56 °C, 32 seconds, collect the fluorescent signal in FAM, HEX, ROX and Cy5 72 °C,1 minute	40

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 VALUE DETERMINATION

After the run of qPCR, a threshold line should be carefully set up to allow accurate Cq (shown in **Figure 3**). 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.

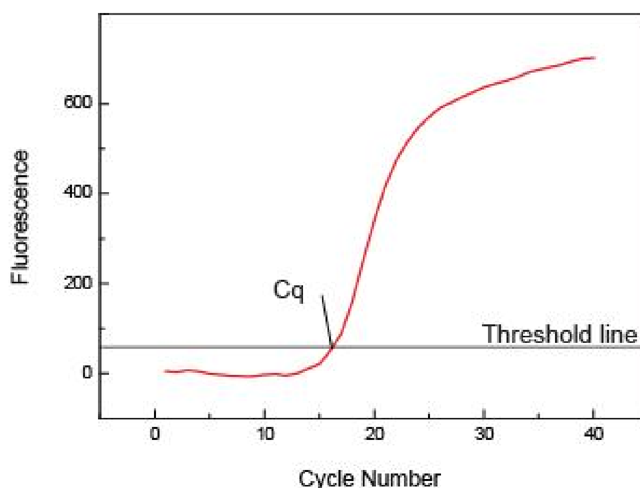


Figure 3. Cq is the intersection between the amplification curve and the threshold line.

8.2 DD H_2O

The Cq values in any channels should be above 35. If not, the run is invalid and should be repeated.

8.3 POSITIVE CONTROL

Signal should be detected in all positive controls

- **P/R L POSITIVE CONTROL,** the Cq values of FAM channel, HEX channel and CY5 channel should be less than 25.
- **P/R S POSITIVE CONTROL**, the Cq values of ROX channel and CY5 channel should be less than 25.
- **P/R V POSITIVE CONTROL**, the Cq values of FAM channel and CY5 channel should be less than 25.
- If not, the run is invalid and should be repeated.

SAMPLES

- The Cq values of Cy5 channels should be less than 25.
- Cq values for positive sample signals other than Cy5 should be below 30. Cq values above 30 might be

non-specific amplification (shown in **Figure 4**). Repeat the test with fresh RNA, if the second test is positive, the sample is positive for the corresponding fusion gene. Other diagnostic techniques are highly recommended to confirm the results from positive tests with Ct values above 30.

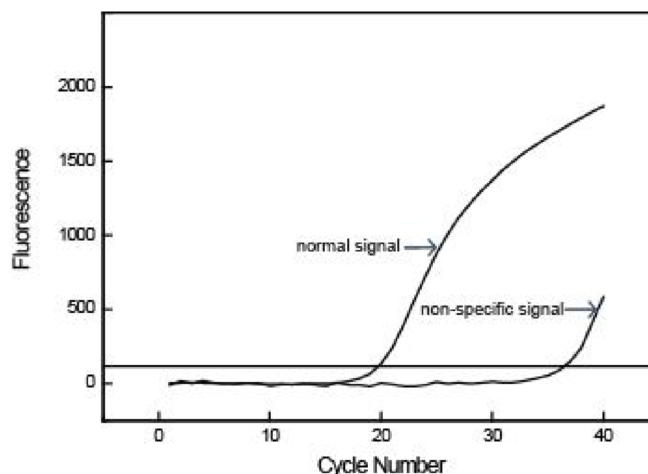


Figure 4. Non-specific signal in sample

- The fusion gene type for positive samples should be interpreted according to **Table 5**.

Table 5. Interpretation Table

Fluorescence Signals	Results
FAM+HEX+Cy5	PML-RARα L
ROX+Cy5	PML-RARα S
FAM+Cy5	PML-RARα V
Cy5	Not PML-RARα L, PML-RARα S, PML-RARα V splice variant

9. LIMITATION OF THE METHOD

- The kit can only differentiate PML-RARα L, PML-RARα S and PML-RARα V splice variants, other fusion genes or splice variants are not covered.
- The Cq values cannot be used for quantification of the fusion transcripts level.
- The low level of fusion transcripts in samples can result in false negative results because of the kit's limit of detection.

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, and do not use after the expiration date printed on the external box label.
- Use aerosol barrier pipette tips.
- Laboratory workbenches, pipettes and other consumables must be cleaned with bleach regularly.
- Opening qPCR 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:

Catalogue Number

In Vitro Diagnostic Medical Device

Manufacturer

Production Date

CE Mark

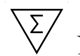
Store between -25 °C and -18 °C

Expiration Date

Do Not Reuse

CAUTION

Consult Instructions for Use

 Number of Tests

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