

Specification

16/48 tests/kit

Intended Use

This kit is used to detect ribonucleic acid (RNA) of the fusion gene PML-RARα (L-type) and ABL1 in peripheral blood samples from patients with acute promyelocytic leukemia (APL/AML-M3) in acute myelogenous leukemia (Acute Myelogenous Leukemia, AML).

This kit is not intended for the diagnosis of leukemia.

Principle of Detection

This kit is designed with specific primers and TaqMan fluorescent probes targeting the PML-RARα (L-type) fusion gene and the ABL1 gene. Using RNA from patient peripheral blood as the template, the assay is performed on a digital PCR all-in-one system to detect the PML-RARα (L-type) fusion gene.

ABL1 is used as an internal control to monitor the extraction and detection processes. Meanwhile, by incorporating UDG, the kit can prevent false-positive results caused by environmental contamination.

Contents and Specification

No.	Content	Amount		Main Component
		16 Tests	48 Tests	
1	Reverse Transcription PCR Mix	176μL×1	550μL×1	One-step RT-dPCR Buffer, dNTP/dUTP Mix, MgCl ₂ , FAM Reference Dye
2	Primer Probe Mix	16μL×1	50μL×1	Primers and Probes
3	Enzyme Mix	32μL×1	100μL×1	Taq DNA Polymerases, Reverse Transcriptase, Uracil-DNA Glycosylase
4	Positive Control	15μL×1	15μL×1	Pseudovirus RNA
5	Negative Control	15μL×1	15μL×1	Negative cell Line RNA
	DNase/RNase-Free Water	500μL×1	500μL×1	DNase/RNase-Free Water

Note:

- The components in different lots of kits cannot be mixed.
- The kit does not contain 8-tube strips. Axygen 0.2mL 8-tube strips (Cat. No. PCR-0208-C) are recommended.
- Equipment and Reagents Not Included but Required for Testing:
Biological safety cabinet, benchtop centrifuge, vortex mixer, nuclease-free centrifuge tubes, 8-tube strips, pipette tips, nucleic acid extraction reagents (Manufacturer: Suzhou Sniper Medical Technologies Co., Ltd).

Storage

- The kit should be stored at -20°C ±5°C with a shelf life of 12 months. Use within the expiration date indicated on the kit.
- Reagents stored at 2-8°C are stable for 7 days; stored at -20°C ±5°C are stable for 12 months. Reagents should not be repeatedly frozen and thawed more than 5 times.

Compatible Instrument

Applicable for the All-in-one Digital PCR Device manufactured by Suzhou Sniper Medical Technologies Co., Ltd.

Sample Requirements

- Applicable Sample Type: Peripheral blood samples.

Suzhou Sniper Medical Technologies Co., Ltd.

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Please visit www.sniper-tech.com for a complete listing of recommended products.

2. **Sample Collection:**
Use a disposable sterile syringe to collect 2-10 mL of venous blood from the subject. Inject the blood into an EDTA anticoagulant-containing blood collection tube. Immediately and gently invert and mix the tube 5-10 times to ensure uniform anticoagulant-blood mixing.
3. **Sample Storage and Transportation**
 - a) Peripheral blood samples can be processed for RNA extraction and detection immediately or stored at 4°C for no more than 24 hours.
 - b) For long-term storage, RNA samples should be kept at -70°C and avoid more than 2 freeze-thaw cycles.

Methods

1. Nucleic Acid Extraction

1. This kit does not contain nucleic acid extraction components. It is recommended to use Nucleic Acid Extraction Reagent (Manufacturer: Suzhou Sniper Medical Technologies Co., Ltd.; Cat. No.: DT007048C) or TRIzol Reagent (Manufacturer: Thermo Fisher Scientific (China) Co., Ltd.; Cat. No.: 15596018 or 15596026) for RNA extraction from samples (follow the corresponding instructions for use).
2. As RNA in peripheral blood is prone to degradation, it is recommended to perform RNA extraction within 24 hours after obtaining the peripheral blood sample. RNA concentration and purity may affect the test results. Therefore, it is recommended (but not mandatory) to measure RNA concentration and purity using a micro-spectrophotometer, ensuring RNA concentration > 40 ng/μL and OD260/OD280 > 1.6.
3. RNA input amount affects assay sensitivity. It is recommended to calculate the RNA input based on RNA concentration and meet an input of 300–500 ng/reaction (dilute RNA to 100 ng/μL for use). Requirements are as follows:

Item	Requirement
Peripheral blood volume	2-10mL
RNA concentration	>40ng/μL
RNA purity	OD260/OD280>1.6
RNA input	300-500ng/reaction

2. Reaction Mix

1. Take out all the components of the kit in the reagent preparation area and place them on ice. After the reagents are completely thawed, briefly centrifuge them and then put them back on ice for later use.
2. **Preparation of the reaction solution:**
Calculate the required volume of each reaction component according to the number of samples to be tested. The reaction system designed for this kit is 22μL. Prepare the reaction solution as follows:

Reaction Component	Volume
Reverse Transcription PCR Mix	11μL
Primer Probe Mix	1μL
Enzyme Mix	2μL

After thoroughly mixing the above reaction solution, briefly centrifuge it and dispense it into 8-tube strips at 14μL per tube.

3. **Sample addition**
 - i. **Control samples:** Take 3μL each of the positive and negative controls and add them to separate 8-tube strips respectively. Then add 5μL of nuclease-free water to make up a final reaction system of 22μL.
 - ii. **Samples to be tested:** Calculate the volume X μL of the RNA sample to be added according to an amount of 300-500ng. Then add $(8-X)$ μL of nuclease-free water to make up a final reaction system of 22μL.
 - iii. Tightly close the tube caps, vortex - mix for 5 - 10 s, and briefly centrifuge to ensure there are no air bubbles in the 8-tube strips.

Note:

1. A negative control and a positive control should be set up for each test.
2. The number of reactions for each run on the instrument should be a multiple of 4 (i.e., 4, 8, 12, 16 reactions). If the number is insufficient, the remaining reactions can be replaced with 22μL of nuclease-free water.

3. Program Setup

1. Place the corresponding consumables according to the *Sniper All-in-one Digital PCR Device User Manual*.
2. Click "Method" to configure the amplification program as follows:

	Step	Temperature	Time	Cycle
1	UDG anti-contamination	25°C	300s	1
2	Reverse transcription & pre-denaturation	50°C	1800s	1
3	Enzyme activation	95°C	300s	1
4	Denaturation	96°C	15s	5
5	Annealing/Extension	60°C	20s	
6	Denaturation	96°C	15s	40
7	Annealing/Extension	63°C	20s	
8	Final extension & fluorescence acquisition	72°C	120s	1

Note: The temperature ramp rate should be set to 2°C/s throughout the entire amplification program.

3. Fluorescence channel selection:
PML-RARα (L-type): VIC
ABL1: ROX
Droplet recognition channel: FAM

4. Result Analysis

1. Result review and threshold line determination:
 - i. After dPCR reaction completion, navigate to "Analysis Plot" → "1D dot Plot" or "2D dot Plot" → "Well Table" to view amplification results for each well.
 - ii. Select the reaction well of the positive control, choose "All", and set the threshold line at the 1/3 position between the negative and positive droplet clusters (closer to the negative cluster). Click "Apply".
 - iii. Verify all reaction wells to ensure the threshold line does not intersect any droplet clusters.
 - iv. If the threshold line fails to clearly separate negative/positive clusters or intersects clusters for a sample, adjust the threshold line individually for that sample using the method in step ii (note: select "Single").
 - v. Positive droplet count and concentration results will be generated based on the threshold line.
2. Result export:
Insert a USB flash drive into the instrument's external USB port. Open the software's "Analysis" menu, click the "Export" button, and save the test results.
3. Result calculation:
The kit reports results as Ratio, calculated as:

$$Copies = \frac{Valid\ Droplets \times 0.6(nl)}{1000} \times C$$

$$Ratio = \frac{PML - RAR\alpha\ (L - type)\ Copy\ Number}{ABL1\ Copy\ Number} \times 100\%$$

Note:

Valid Droplet: Valid droplet count.

C: Instrument detection value (copy/μL).

5. Quality Control

1. Quality control for negative and positive controls:
The following criteria must be met; if not, the experiment should be repeated:

Control Name	Expected Result
Positive Control	PML-RARα (L-type) Ratio=10% (acceptable range: $\pm 30\%$ of target value)
Negative Control	PML-RARα (L-type) copy number ≤ 1

- Valid droplet count requirement:
Each reaction well must generate $\geq 18,000$ valid droplets. If not met, the reaction well should be retested.
- Result validity:
Proceed to analysis if all requirements (5.1 and 5.2) are satisfied. If 5.1 is met but some reaction wells fail to meet 5.2, only retest the non-compliant wells.

Positive Cut-off Value

If the quality control requirements are met, a result is considered positive when the number of PML-RARα (L-type) positive droplet ≥ 1 . If the PML-RARα (L-type) positive droplet count < 1 under valid quality control, the result is negative.

Interpretation of Test Results

Test results are interpreted as follows under compliance with quality control requirements (5.1–5.3):

Test Result	Report Result	Result Interpretation
PML-RARα (L-type) copy number = 0	Negative	No PML-RARα (L-type) detected in the sample.
Ratio $< 0.002\%$	Substance is present	The result below the kit's limit of detection and should not be used as the basis for judgement.
$0.002\% \leq \text{Ratio} < 0.0032\%$	Positive	PML-RARα (L-type) fusion gene detected; for reference only. There is a certain probability of no value upon retest for this portion of sample
Ratio $\geq 0.0032\%$	Positive	PML-RARα (L-type) fusion gene detected.

Note:

- When ABL1 copies $\leq 10,000$, investigate the cause or increase sample input and retest.
- When $10,000 < \text{ABL1 copies} \leq 32,000$ and PML-RARα (L-type) copies = 0, ABL1 copies are too low; increase RNA input and retest.
- When ABL1 copies $> 140,000$ and PML-RARα (L-type) copies > 0 , results may be reported; however, ABL1 copies exceed the linear range, which affects quantitative accuracy. Reduce RNA input and retest.

Product Performance Specifications

1. Linearity

Test enterprise linear reference materials and fit the measured Ratio values to obtain the linear correlation coefficient r , meeting $|r| \geq 0.98$.

2. Accuracy

Test enterprise accuracy reference materials; the deviation between the mean measured concentration and theoretical concentration is within $\pm 0.5 \text{ Log}$.

3. Concordance Rate

Test enterprise negative and positive reference materials; concordance rate 100%.

4. Limit of Blank (LoB)

Test enterprise LoB reference materials 5 times; 100% negative.

5. Limit of Detection (LoD)

Test enterprise LoD reference materials 5 times; 100% positive.

6. Limit of Quantification (LoQ)

Single-well testing of enterprise LoQ reference materials 5 times: 100% detectable, and coefficient of variation (CV) of results $\leq 10\%$.

7. Precision

Test enterprise precision reference materials 5 times: CV of results $\leq 10\%$.

8. Specific Detection

Anti-interference: hemoglobin (≤ 100 g/L), sodium heparin (≤ 24 mg/L), total bilirubin (≤ 257 μ M), EDTA (≤ 5 mg/mL), triglycerides (≤ 5.6 mM), cholesterol (≤ 6.47 mM) show no impact on detection. Cross-reactivity: no cross-reaction with CML or ALL-P190, CML-P210, CML-P230, AML1-ETO, PML-RAR α (S-type), PML-RAR α (V-type), CBF β /MYH11, TEL/AML1, E2A/PBX1, MLLT3/MLL, MLL/AF4(e9e5), MLL/AF4(e10e4).

Precautions

1. This product is for research use only. Read the instruction carefully before use.
2. Familiarize yourself with the operation and precautions of all instruments before testing. Implement quality control for each experiment.
3. Follow strict PCR gene amplification laboratory management specifications. Failure to control cross-contamination during sample processing may result in false positive results.
4. Avoid repeated freeze-thaw cycles of reagents. Mix thoroughly and briefly centrifuge before use.
5. Test results depend on sample collection, processing, transportation, and storage quality. Any errors in these steps may lead to inaccurate results.

Basic Information

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Instruction Approval and Revision Date

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