

Leukemia Fusion Gene PML-RAR α (L Type, V Type) Nucleic Acid Detection Kit (Digital PCR Method)

Instructions for Use — English Translation

PACK SIZE

16 or 48 tests per kit.

INTENDED USE

This kit is used for the quantitative detection of ribonucleic acid (RNA) from the PML-RAR α L-type and V-type fusion genes and ABL1 in peripheral blood from patients with acute promyelocytic leukemia (APL/AML-M3), a form of acute myeloid leukemia (AML). This kit is not intended for the diagnosis of leukemia.

PRINCIPLE OF THE TEST

The kit contains specific primers and TaqMan fluorescent probes for the PML-RAR α L-type and V-type fusion genes and ABL1. RNA from patient peripheral blood is tested using an integrated digital PCR instrument to quantify the PML-RAR α L and V types. ABL1 serves as a control for extraction and testing. The addition of UDG helps prevent false-positive results caused by environmental contamination.

KIT COMPONENTS

No.	Component	16 tests	48 tests	Main constituents
1	RT-PCR Mix	176 μ L $\times$ 1 tube	550 μ L $\times$ 1 tube	One-step RT-dPCR Buffer; dNTP/dUTP Mix; MgCl ₂ ; FAM Reference Dye; RNase Inhibitor, etc.
2	Primer/Probe Mix	16 μ L $\times$ 1 tube	50 μ L $\times$ 1 tube	Primers and probes
3	Enzyme Mix	32 μ L $\times$ 1 tube	100 μ L $\times$ 1 tube	Taq DNA polymerases; reverse transcriptase; RNase inhibitor; uracil-DNA glycosylase
4	Positive Control	15 μ L $\times$ 1 tube	15 μ L $\times$ 1 tube	Pseudoviral RNA
5	Blank Control	500 μ L $\times$ 1 tube	500 μ L $\times$ 1 tube	DNase/RNase-free water

1. Do not interchange components from kits of different lot numbers.
2. Eight-tube strips are not included. Recommended: Axygen 0.2 mL eight-tube strips, cat. no. PCR-0208-C.

Equipment and reagents required but not supplied

Biosafety cabinet, benchtop centrifuge, vortex mixer, nuclease-free centrifuge tubes, eight-tube strips, pipette tips, and nucleic acid extraction reagent.

STORAGE CONDITIONS AND SHELF LIFE

Store the kit at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. Shelf life is 12 months; use before the expiry date printed on the kit. Reagents may be kept at $2-8^{\circ}\text{C}$ for up to 7 days or at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for up to 12 months. Do not exceed five freeze-thaw cycles.

COMPATIBLE INSTRUMENT

An integrated digital PCR instrument manufactured by Suzhou Sniper Medical Technologies Co., Ltd.

SPECIMEN REQUIREMENTS

1. Specimen type: Peripheral blood.
2. Collection: Collect 2–10 mL of venous blood with a disposable sterile syringe and transfer it to an EDTA anticoagulant collection tube. Immediately invert the tube gently 5–10 times to mix the anticoagulant and blood thoroughly.
3. Storage and transport: Extract and test RNA from peripheral blood immediately, or store the blood at $2-8^{\circ}\text{C}$ for no more than 24 hours. For long-term storage, keep extracted RNA at -70°C and avoid more than two freeze-thaw cycles.

TEST PROCEDURE

1. Nucleic acid extraction

1.1 Nucleic acid extraction components are not supplied. TRIzol Reagent (Thermo Fisher Scientific [China] Co., Ltd.; cat. no. 15596018 or 15596026) is recommended for sample RNA extraction. Follow the reagent manufacturer's instructions.

1.2 RNA in peripheral blood degrades readily; extract RNA within 24 hours of collection. RNA concentration and purity affect results. Measurement with a microvolume spectrophotometer is recommended, though not mandatory. Ensure RNA concentration $>40\text{ ng}/\mu\text{L}$ and $\text{OD}_{260}/\text{OD}_{280} >1.6$.

1.3 RNA input affects sensitivity. Calculate the amount from the RNA concentration and use 300–500 ng per reaction. Dilute RNA to 100 ng/ μL before use. Requirements:

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

2. Amplification and detection

2.1 In the reagent preparation area, remove the kit components and place them on ice. After complete thawing, centrifuge briefly and return to ice.

2.2 Prepare the reaction mix. Calculate the required volume of each component from the number of positive controls, blank controls, and samples. The final reaction volume is 22 μL. Prepare the following mix per reaction:

Reaction component	Volume per reaction
RT-PCR Mix	11 μL
Primer/Probe Mix	1 μL
Enzyme Mix	2 μL

Mix the reaction mix thoroughly, spin briefly, and dispense 14 μL into each eight-tube strip well.

2.3 Add samples and controls. Controls: Add 3 μL of positive or blank control to separate wells and add 5 μL nuclease-free water to each, giving 22 μL total. Samples: Calculate X μL of RNA to provide 300–500 ng, and add (8 – X) μL nuclease-free water to give 22 μL total. Cap tightly, vortex for 5–10 seconds, spin briefly, and ensure there are no bubbles.

Note 1: Include a blank control and positive control in every run. Note 2: Load a multiple of four reactions (4, 8, 12, 16, etc.). For unused positions, substitute 22 μL nuclease-free water.

3. Sample setup and amplification program

3.1 Place the appropriate consumables as described in the Sniper Integrated Digital PCR Instrument User Manual.

3.2 Select “Method” and set the following program:

Step	Operation	Temperature	Time	Cycles
1	UDG decontamination	25 °C	300 s	1
2	Reverse transcription and predenaturation	50 °C	1,800 s	1
3	Enzyme activation	95 °C	300 s	1
4	Denaturation	94 °C	30 s	5*
5	Annealing/extension	60 °C	60 s	5*
6	Denaturation	94 °C	30 s	40*
7	Annealing/extension	63 °C	60 s	40*
8	Final extension and fluorescence acquisition	72 °C	120 s	1

* The source table shows five cycles spanning steps 4–5 and 40 cycles spanning steps 6–7.

Fusion-gene fluorescence channels: VIC and Cy5. ABL1 fluorescence channel: ROX. Set the heating rate to 2 °C/s throughout the amplification program.

3.3 Under “Fluorescence Channel,” select VIC and Cy5 for the fusion genes, ROX for ABL1, and FAM as the droplet identification channel.

4. Result analysis

4.1 Viewing results and setting thresholds:

- After dPCR, open “Analysis Plots” → “1D Scatter Plot” or “2D Scatter Plot” → “Well Table” to view the amplification results for each well.
- Select the positive-control well and “All.” Set the threshold line one-third of the way between the negative and positive droplet clusters, closer to the negative cluster, and click “Apply.”
- Check every reaction well and ensure the threshold line does not pass through any droplet cluster.
- If the threshold does not adequately separate the negative and positive clusters of a sample or crosses a cluster, set a separate threshold for that sample using the method in step b, selecting “Single.”
- Use the threshold to obtain the positive droplet count, concentration, and other results.

4.2 Exporting results: Insert a USB drive into the instrument’s USB port. In the software “Analysis” menu, click “Export” to export the run results.

4.3 Calculations:

PML-RARα L-type copies in a 22 μL reaction = VIC = Cy5 instrument result (copies/μL) × 22 μL.

PML-RARα L-type copies in a 22 μL reaction = Cy5 instrument result (copies/μL) × 22 μL (provided VIC = 0). [Source labels this equation "L type"; verify with manufacturer.]

Sample concentration (copies/mL) = copy number ÷ sample input volume × 1,000.

Ratio = PML-RARα L-type or V-type copy number ÷ ABL1 copy number.

5. Quality control

5.1 Blank and positive controls must meet the criteria below. If either fails, repeat the run:

Control	Expected result
Positive Control	PML-RARα L-type and V-type ratio: 100% ± 5%
Blank Control	PML-RARα L-type and V-type copies ≤1; ABL1 copies ≤10

5.2 Total droplets: Each reaction well must generate at least 15,000 valid droplets. Retest any well that fails.

5.3 If all criteria are met, the run is valid and may be analyzed. If 5.1 is met but any well fails 5.2, retest only the failing well(s).

POSITIVE RESULT CRITERIA

When kit quality-control requirements are met, a result is PML-RARα L-type positive if VIC = Cy5 positive droplets and the number of positive droplets is ≥1. A result is PML-RARα V-type positive if the VIC positive droplet count is 0 and the Cy5 positive droplet count is ≥1. When quality-control requirements are met and the number of VIC and Cy5 positive droplets is <1, the result is negative.

INTERPRETATION OF RESULTS

1. Provided the quality-control requirements in 5.1–5.3 are met, interpret results as follows:

Test result	Report	Interpretation
PML-RARα L-type and/or V-type copies = 0	Negative	No PML-RARα L-type and/or V-type fusion gene detected in the sample.
Ratio <0.00002	Substance present; cannot be quantified	Result is outside the kit's lower detection limit and must not be used as a basis for interpretation.
0.00002 ≤ Ratio <0.000032	Report the ratio value	PML-RARα L-type or V-type fusion gene detected, but the result is outside the quantitative linear range and is for reference only. Some such samples may yield no numeric value on retesting.
Ratio ≥0.000032	Report the ratio value	PML-RARα L-type or V-type fusion gene detected.

Notes:

- If ABL1 copies ≤10,000, report the result as invalid. Investigate the cause or retest with a larger sample amount.
- If 10,000 < ABL1 copies ≤32,000 and PML-RARα L-type or V-type copies = 0, the ABL1 copy number is too low. Retest with increased RNA input.
- If ABL1 copies >140,000 and PML-RARα L-type or V-type copies >0, the result may be reported, but the ABL1 copy number exceeds the linear range and may affect quantitative accuracy. Retesting with less RNA may be performed.

PRODUCT PERFORMANCE

- Linear range. The kit was tested with the manufacturer's linearity reference materials. A fitted curve of measured ratios gives a linear correlation coefficient |r| ≥0.98.
- Accuracy. With the manufacturer's accuracy reference materials, the difference between the mean measured concentration and theoretical concentration is within ±0.5 log.
- Agreement. Agreement with the manufacturer's negative reference materials is 100%; agreement with the positive reference materials is 100%.
- Limit of blank. In five tests of the manufacturer's blank-limit reference material, 100% of results were negative.
- Limit of detection. In five tests of the manufacturer's detection-limit reference material, 100% of results were positive.
- Limit of quantification. In five single-well tests of the manufacturer's quantification-limit reference material, the detection rate was 100% and the coefficient of variation (CV) of measured results was ≤10%.
- Precision. In five tests each of the manufacturer's precision reference materials, the CV of measured results was ≤10%.
- Specificity. Interference: Hemoglobin (≤100 g/L), sodium heparin (≤24 mg/L), total bilirubin (≤257 μM), EDTA (≤5 mg/mL), triglycerides (≤5.6 mM), and cholesterol (≤6.47 mM) had no effect on detection at the concentrations tested.

Cross-reactivity: No cross-reaction was found with CML or ALL-P190, CML-P210, CML-P230, AML1-ETO, PML-RARα (S type), CBFβ/MYH11, TEL/AML1, E2A/PBX1, MLLT3/MLL, MLL/AF4(e9e5), or MLL/AF4(e10e4).

PRECAUTIONS

1. For research use only. Read these instructions carefully before use.
2. Before testing, understand the operation and precautions for all instruments used, and perform quality control for every experiment.
3. Manage the laboratory strictly according to PCR gene-amplification laboratory requirements. Failure to control cross-contamination during specimen handling may cause false-positive results.
4. Avoid repeated reagent freeze-thaw cycles. Mix thoroughly and spin briefly before use.
5. Results depend on the quality of specimen collection, handling, transport, and storage. Errors at any of these stages may cause inaccurate results.

MANUFACTURER INFORMATION

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IFU APPROVAL AND REVISION DATE

Source IFU approval date: 27 September 2023.

Translation note: This English version is translated from the supplied Chinese CUM-R10V (V1.0.1) IFU. The source's second copy-number equation in section 4.3 repeats the "L type" label despite specifying VIC = 0. The label is retained above and should be confirmed with the manufacturer before use.