

Specification

16/48 tests/kit

Product Description

This RUO kit is intended for the quantitative detection of BCR-ABL1 (p230) fusion gene RNA and ABL1 RNA in peripheral blood samples from individuals with leukemia.

The Philadelphia chromosome (Ph), resulting from the chromosomal translocation t(9;22)(q34;q11), is a hallmark of chronic myeloid leukemia (CML). At the molecular level, this translocation leads to the formation of the BCR-ABL1 fusion gene, which involves the BCR gene on chromosome 22 and the ABL1 gene on chromosome 9. Depending on the breakpoint in the BCR gene, the fusion transcript can be classified into three types: m-BCR (p190), M-BCR (p210), and u-BCR (p230). The p230 fusion protein is encoded by a BCR gene rearrangement associated with specific variants of chronic myeloid leukemia (CML), including CML with excessive neutrophils (CML-N) and CML with excessive thrombocytosis (CML-T).

This kit is not intended for the diagnosis of chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia. The results obtained are for research purposes only and are not to be used as the sole basis for clinical assessment. Clinical interpretation should be based on a comprehensive evaluation by qualified professionals, taking into account the patient's clinical presentation and other laboratory findings

For BCR-ABL1 (p230) fusion gene and ABL1 gene, specific primers and fluorescent probes are designed. Using RNA extracted from peripheral blood of the patient as the template, detection is performed via All-in-one digital PCR instrument to achieve identification of the BCR-ABL1 (p230) fusion gene. The kit uses ABL1 as an internal control to monitor the extraction and detection processes. Additionally, the inclusion of UDG (uracil-DNA glycosylase) prevents false positives caused by environmental contamination.

Contents and Specification

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

Note:

- Components from different lot numbers of the kit should not be mixed.
- The kit does not include 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.

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, they are stable for 12 months. However, they should not be subjected to more than 5 freeze-thaw cycles.

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Compatible Instrument

Suitable for All-in-one Digital PCR Instruments manufactured by Suzhou Sniper Medical Technology Co., Ltd.

Sample Requirements

1. Applicable Sample Type: Peripheral blood samples.
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 -80°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 Blood Total RNA Extraction Kit (Manufacturer: Suzhou Sniper Medical Technology 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 (extraction methods refer to the respective instructions).
2. Due to the susceptibility of RNA in peripheral blood to degradation, RNA extraction should be performed within 24 hours of sample collection. The concentration and purity of RNA may affect test results. While optional, it is recommended to measure RNA concentration and purity using a micro-volume spectrophotometer, ensuring RNA concentration >40 ng/μL and an OD260/OD280 ratio >1.6.
3. The RNA input amount impacts detection sensitivity. Calculate the RNA input based on concentration to ensure 300-500 ng per reaction (dilute RNA to 100 ng/μL for use). Specific requirements are as follows:

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

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
RT-PCR Master Mix	11 μL
Primer Probe Mix	1 μL
Enzyme Mix	2 μL

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 300ng-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 Instrument 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:
BCR-ABL1 (p230): 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" → "Plate Map" 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. Sample selection
Samples with ABL1 copy number >10,000 should be selected for testing.
When the ABL1 copy number is ≤10,000, detection sensitivity may be affected. In such cases, the input amount can be increased or pooled wells may be used for testing.
4. Result calculation:
Nb Droplet: Valid droplet count.

C: Instrument detection value (copy/ μ L).

$$\text{Copy Number} = \frac{\text{Nb Droplets} \times 0.6(\text{nl})}{1000} \times C$$

$$\text{Ratio} = \frac{\text{BCR} - \text{ABL1 (p230) Copy Number}}{\text{ABL1 Copy Number}}$$

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	BCR-ABL1 (p230) Ratio: 10% (acceptable range: $\pm 30\%$ of target value)
Negative Control	BCR-ABL1 (p230) copy number < 1

2. Valid droplet count requirement:
Each reaction well must generate $\geq 18,000$ valid droplets. If not met, the reaction well should be retested.
3. 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 BCR-ABL1 (p230) positive droplets ≥ 1 . If the positive droplets 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
BCR-ABL1 (p230) copy number = 0	Negative	No BCR-ABL1 (p230) fusion gene detected in the sample.
Ratio $< 0.002\%$	Fusion gene detected, but not quantifiable	Below the lower limit of detection; result should not be used for interpretation.
$0.002\% < \text{Ratio} < 0.0032\%$	Fusion gene detected, but not quantifiable	BCR-ABL1 (p230) fusion gene detected; result is for reference only. Retesting may yield non-quantifiable results.
Ratio $\geq 0.0032\%$	Positive, Ratio Value (%)	BCR-ABL1 (p230) fusion gene detected.

Note:

1. The linear range for copy number detection on the compatible digital PCR system is 3–150,000 copies. It is recommended to analyze results within this range.
2. If ABL1 copy number $\leq 32,000$ and BCR-ABL1 copy number = 0, the ABL1 copy number may be too low. Consider increasing RNA input and retesting.
3. If ABL1 copy number $> 150,000$ and BCR-ABL1 (p230) copy number > 0 , results may still be reported. However, since ABL1 exceeds the linear range, quantification accuracy may be affected. Consider reducing RNA input and retesting.
4. For pooled wells, the ratio is calculated as:
(Total BCR-ABL1 copies) / (Total ABL1 copies)

Product Performance Specifications

Concordance Rate	Testing with manufacturer-provided positive and negative reference materials showed
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	a 100% concordance rate.
Limit of Blank (LoB)	All 5 replicates of blank reference material tested negative (100% negative rate).
Limit of Detection (LoD)	All 5 replicates of reference material at Ratio: 0.002% tested positive.
Limit of Quantitation (LoQ)	All 5 replicates of reference material at Ratio: 0.0032% tested positive, with the absolute value of the coefficient of variation (CV) for Ratio (%) $\leq 10\%$.
Precision	Testing of precision reference material in 5 replicates resulted in a CV for Ratio (%) $\leq 10\%$.
Specificity	Interference Testing The kit showed no interference from the following endogenous substances at specified concentrations: Hemoglobin (≤ 100 g/L), Heparin sodium (≤ 24 mg/L), Total bilirubin (≤ 257 μM), EDTA (≤ 5 mg/mL), Triglycerides (≤ 5.6 mM), Cholesterol (≤ 6.47 mM). Cross-reactivity Testing No cross-reactivity was observed when tested with CML-P210, CML-P190, AML1/ETO, PML/RARA, CBFβ/MYH11, TEL/AML1, E2A/PBX1, MLLT3/MLL, MLL/AF4 (e9e5), and 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

Approval Date:

Revision Date: July 10, 2025