

Specifications

16 / 48 tests / kit

Intended Use

The BCR-ABL1 (p210) %IS Kit (Digital PCR Method) is an in vitro nucleic acid amplification test for the quantitation of BCR-ABL1 and ABL1 transcripts in total RNA from whole blood of diagnosed t(9;22) positive Chronic Myeloid Leukemia (CML) adult patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2. By calculating the ratio of BCR-ABL1 to ABL1 copy numbers and converting it to the International Scale percent ratio (%IS), the report is expressed in terms of Molecular Reduction level (MR).

The test does not differentiate between e13a2 or e14a2 fusion transcripts and does not monitor other rare fusion transcripts resulting from t(9;22). This test is not intended for the diagnosis of CML.

Test Principle

This kit is designed for BCR-ABL1 fusion gene (p210) and ABL1 gene, with specific primers and specific fluorescence probes. The test process is divided into three parts. The first part is to extract ribonucleic acid (RNA) from peripheral blood of patients. The second part is to detect BCR-ABL1 fusion gene (p210) and ABL1 internal reference gene in RNA samples by RT-dPCR reaction solution on the Sniper Digital PCR All-in-One System, and the third part is to analyze the results.

Meanwhile, the kit can prevent the occurrence of false positive result due to cross-contamination by adding UDG.

Main Components and Specifications

Table 1

No.	Component	16 Tests	48 Tests	Main Composition
1	One-step RT-dPCR Master Mix	220 μ L $\times$ 1tube	660 μ L $\times$ 1tube	One-step RT-dPCR Buffer、dNTP/dUTP Mix、MgCl ₂ 、FAM Reference Dye、RNase Inhibitor
2	BCR-ABL1 Primer Probe Mix	20 μ L $\times$ 1tube	60 μ L $\times$ 1tube	Primer, Probe
3	BCR-ABL1 Enzyme Mix	40 μ L $\times$ 1tube	120 μ L $\times$ 1tube	Taq DNA Polymerases、Reverse Transcriptase、RNase Inhibitor、Uracil-DNA Glycosylase
4	Positive Control 1	15 μ L $\times$ 1tube	15 μ L $\times$ 2tube	A mixture of K562 cell line RNA and HL60 cell line RNA at 10% IS.
5	Positive Control 2	15 μ L $\times$ 1tube	15 μ L $\times$ 2tube	A mixture of K562 cell line RNA and HL60 cell line RNA at 0.1% IS.
6	Negative Control	15 μ L $\times$ 1tube	15 μ L $\times$ 2tube	HL60 cell line RNA
7	Nuclease Free Water	1000 μ L $\times$ 1tube	1000 μ L $\times$ 1tube	DNase/RNase-Free water

※ The components of different batches of the reagent kit cannot be cross used.

Materials required but not provided

Biosafety cabinet, bench-top centrifuge, vortex oscillator, nuclease-free centrifuge tube, PCR reaction 8 tubes per strip, pipette tip, nucleic acid extraction reagent (Whole Blood Nucleic Acid Extraction Reagent, Art. No. DT007048C, Manufacturer: Suzhou Sniper Medical Technologies Co., Ltd.).

Storage Conditions and Shelf Life

The reagent kit should be stored at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and has a shelf life of 12 months. Please use it within the expiration date indicated on the kit. The reagents can be used for 7 days at $2-8^{\circ}\text{C}$ or 12 months at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ and cannot be frozen and thawed more than 5 times.

Applicable Instruments

Applicable for the digital PCR instruments manufactured by Suzhou Sniper Medical Technologies Co., Ltd.

Sample Requirements

■ Applicable sample type

Peripheral blood samples.

■ Sample collection

Use a disposable sterile syringe to draw 2-10 mL of venous blood from the subject, inject it into the blood collection tube containing EDTA anticoagulant, gently reverse and mix the blood collection tube for 5-10 times right away, so that the anticoagulant is evenly mixed with venous blood.

■ Sample storage

Peripheral blood samples can be immediately extracted and tested for RNA or stored at 4°C for no more than 24 hours; RNA samples should be stored in a -80°C refrigerator for a long time if necessary, and repeated freezing and thawing for more than 2 times should be avoided.

Test Method

1. Preparation

1) The kit contains no nucleic acid extraction components, and it is recommended to use Whole Blood Nucleic Acid Extraction Reagent (Manufacturer: Suzhou Sniper Medical Technologies Co., Ltd. Catalog Number: DT007048C) for sample RNA extraction (refer to its instructions for extraction method). Controls are not involved in extraction.

2) Since RNA in peripheral blood is easy to degrade, it is recommended to extract RNA within 24 hours after obtaining peripheral blood samples; The concentration and purity of RNA will affect the test results, so it is advised but not mandatory to use a microspectrophotometer to detect the concentration and purity of RNA, which should ensure RNA concentration $>40 \text{ ng}/\mu\text{L}$ and OD260/OD280 ratio > 1.6 .

3) The input amount of RNA will affect the sensitivity of the assay results. Therefore, it is recommended to calculate the input amount of RNA based on the concentration of RNA and meet the input amount of 300-500 ng/reaction (Use nuclease free water to dilute the RNA to the concentration of $100 \text{ ng}/\mu\text{L}$ for future use). The specific requirements are as follows:

Table 2 : Sample quality requirements

Item	Requirements
Peripheral blood volume	2-10 mL
RNA concentration	$>40 \text{ ng}/\mu\text{L}$
RNA purity	OD260/OD280 ratio > 1.6

RNA input

300-500 ng/reaction

2. Amplification and Detection

■ Kit preparation

Take the components out of the kit in the reagent preparation area, place them on ice, wait until the reagents are completely melted, centrifuge briefly and return to ice for later use. Preparation of reaction solution:

The required volume of each reaction component is calculated based on the number of Positive Control 1, Positive Control 2, Negative Control, Blank control and the number of samples to be tested; The reaction system designed for this kit is 22 μ L, and the reaction solution is prepared as follows:

Table 3: Preparation of PCR reaction system

Reaction Composition	Addition per reaction
One-step RT-dPCR Master Mix	11 μ L
BCR-ABL1 Primer Probe Mix	1 μ L
BCR-ABL1 Enzyme Mix	2 μ L
RNA samples	X μ L
Nuclease Free Water	Complete to 22 μ L

The above reaction solution is mixed thoroughly, centrifuged instantaneously, and dispensed into PCR reaction tubes according to 14 μ L/tube.

■ Spiked sample

Add 3 μ L Positive Control 1, Positive Control 2, Negative Control and Nuclease Free Water into PCR amplification tubes, and 5 μ L nuclease-free water was added, to prepare a final reaction system of 22 μ L.

The amount of RNA sample to be tested was calculated as X μ L to 300-500 ng, and (8 - X) μ L nuclease-free water was added to prepare 22 μ L final reaction system.

Cap the tube tightly, shake to mix well for 5-10 seconds, and centrifuge instantaneously to ensure that there is no bubble in the PCR tube.

Note 1: Positive Control 1, Positive Control 2, Negative Control and Blank control, should be set for each test.

Note 2: It should be ensured that the number of reactions should be a multiple of 4 (i.e., 4, 8, 12, and 16 reactions, and the insufficient part can be replaced with 22 μ L of enzyme-free sterile water).

3. Sample Setup and Amplification Program Settings

Arrange the consumables according to the requirements of the digital PCR instrument manual.

Click "Method" to set the amplification procedures as follows:

Table 4

	Procedures	Temperature	Time	Number of cycles
1	UDG anti-contamination	25°C	300s	1

2	Reverse transcription and pre-denaturation	50°C	1800 s	1
3	Enzyme activation	95°C	300 s	1
4	Denaturation	96°C	15 s	5
5	Annealing, extension	60°C	20 s	
6	Denaturation	96°C	15 s	40
7	Annealing, extension	63°C	20 s	
8	Final extension and fluorescence acquisition	72°C	120s	1

※ The ramp rate should be set to 2°C/s during the entire amplification program.

"Target" refers to fluorescence channel, "VIC" is selected for BCR-ABL1 fluorescence channel, and "ROX" is selected for ABL1 fluorescence channel; "FAM" is selected for droplet identification channel.

4. Data Analysis

4.1 Result Viewing and Threshold Setting

- After the dPCR reaction is completed, open the "Analysis Plot" → "1D Scatter Plot" or "2D Scatter Plot" → "Well Table" to view the amplification results of each well.
- Select the reaction wells of the positive control, choose "All", and set the threshold line at 1/2 of the distance between the negative and positive droplet clusters (closer to the negative droplet cluster), then click "Apply".
- Verify all reaction wells to ensure that the threshold line does not pass through the droplet clusters of any well.
- If the set threshold line cannot clearly distinguish between the negative and positive droplet clusters of the test sample or passes through the droplet clusters, the threshold line can be set separately for that test sample by referring to step "b" (note: select "Single").
- The number of positive droplets and concentrations can be obtained based on the set threshold line.

4.2 Result Export:

Insert a USB drive into the external USB interface of the instrument, open the "Analysis" menu of the software, and click the "Export" button to export the test results.

4.3 Result Calculation:

The reporting format of the test results in this kit is International Scale percent ratio (%IS) and Molecular Reduction level (MR)

Calculate the Ratio, International Scale Percent Ratio (%IS) and Molecular Reduction Level (MR) for each sample in the table as follows:

$$Ratio = \frac{BCR - ABL1(copy)}{ABL1(copy)}$$

$$\%IS = Ratio \times 100 \times CF$$

$$MR = \log_{10}\left(\frac{100}{\%IS}\right) = \log_{10}(100) - \log_{10}(\%IS) = 2 - \log_{10}(\%IS)$$

- ※ The copy number calculation method: Copy Number = Instrument Measured Result (copy/μL) × Droplet Volume (nL, read from the instrument) × Nb Droplets (read from the instrument result) / 1000.
- ※ Nb Droplets: effective droplet number.
- ※ CF: Check the label inside the kit for CF value

4.4 Result Report:

Table 5: %IS values and associated MR values

%IS	MR
50	0.3
32	0.5
10	1.0
1	2.0
0.32	2.5
0.1	3.0 (MMR)
0.032	3.5
0.01	4.0
0.0032	4.5
0.002	4.7
0.0012	4.9
0.001	5.0
MMR: Major Molecular Response	

5. Quality Control

5.1 Control sample requirements are as follows, if not met, the experiment should be repeated:

Table 6

Name of the reference substance	Expected result
Blank control	BCR-ABL1 copy≤1 and ABL1 copy≤10
Negative Control	BCR-ABL1 copy≤1
Positive Control 1	MR0.5~MR1.5 (32%IS-3.2%IS)
Positive Control 2	MR2.5~MR3.5 (0.32%IS-0.032%IS)

5.2 Total Droplet Count: Each reaction well should have ≥18,000 effective droplets. If not met, the well should be retested.

5.3 When all the above requirements are met, the current test results are valid and can proceed with the next analysis. When the requirements in 5.1 are met, but the 5.2 requirement is not met for certain wells, only those wells need to be retested.

Cut off Value

When the quality control requirements of this kit are met, if the number of BCR-ABL1(p210) positive droplets ≥1 the test sample is positive; if the number of BCR-ABL1(p210) positive droplets < 1, the test sample is negative.

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Interpretation of Test Results

Under the premise of meeting the quality control requirements, the test results for each mutation are reported and interpreted as follows:

Table 7

Test results	Report results	Explanation of test results
Copy number of BCR-ABL1 was 0	Report: Negative	It indicates that there is no BCR-ABL1 fusion gene in the test sample.
$MR > 4.7$ or $\%IS < 0.002$	Report: BCR-ABL1 fusion gene is detected, but cannot be quantified	The results exceed the lower limit of detection of the kit and cannot be used as a basis for judgment. Retesting can be done using a dual-well method.
$4.5 < MR \leq 4.7$ or $0.002 \leq \%IS < 0.0032$	Report: %IS (MR) value	BCR-ABL1 fusion gene is detected, but the results are beyond the limit of quantitation.
$0.3 \leq MR \leq 4.5$ or $0.0032 \leq \%IS \leq 50$	Report: %IS (MR) value	BCR-ABL1 fusion gene detected and %IS (MR) value measured.
$MR < 0.3$ or $\%IS > 50$	Report: BCR-ABL1 fusion gene is detected, but cannot be quantified	BCR-ABL1 fusion gene is detected.

- ※ When the number of ABL1 copies is $\leq 10,000$, the report sensitivity may be affected. A retest should be performed with increased RNA input.
- ※ In case of $10000 < ABL1 \text{ copy number} \leq 32,000$, and BCR-ABL1 copy number = 0, ABL1 copy number is too low, and a retest should be performed with increased RNA input.
- ※ In the case of ABL1 copy number $> 150,000$ and the BCR-ABL1 copy number > 0 , the ABL1 copy number exceeds the linear range, which will affect the quantitative accuracy. A retest should be performed with reduced RNA input.

Product Performance Characteristics

Linearity/Assay reportable range

By detecting the standardized concentration of the enterprise linear reference samples ranging from 50%IS (MR0.3) to 0.002%IS (MR4.7), each concentration is tested twice, and a linear fit is performed between the theoretical values (MR) and the measured values (MR) to obtain the linear correlation coefficient r , which should meet the requirement of $|r| \geq 0.980$.

Accuracy

The deviation between the mean measured concentration and theoretical concentration is within $\pm 0.5 \text{ Log}$ for the kit by detecting the enterprise accuracy reference substance at the concentration of 10%IS (MR1.0) -0.01%IS (MR4.0).

Negative/Positive Concordance Rate:

The negative/positive concordance rate is 100% when testing the company's negative and positive reference samples.

Limit of blank

By detecting the standardized concentration of the enterprise limit of blank reference samples for 10 tests, all detection results are negative for BCR-ABL1.

Limit of detection

By detecting the standardized concentration of 0.002%IS (MR4.7) of the enterprise limit of detection reference samples for 10 tests, all detection results are positive for BCR-ABL1.

Limit of quantification

By detecting the standardized concentration of 0.0032%IS (MR4.5) of the enterprise limit of quantification reference samples for 10 tests, all detection results are positive for BCR-ABL1, and the precisions were $\leq 10\%$.

Precision/Reproducibility

By detecting the standardized concentration of 10%IS (MR1.0) and 0.01%IS (MR4.0) of the enterprise precision reference samples for 10 tests, all test results are positive for BCR-ABL1. Furthermore, the coefficient of variation (CV) of the measured values (MR) for the 10%IS (MR1.0) reference material is $\leq 5\%$, and the CV of the measured values (MR) for the 0.01%IS (MR4.0) reference material is $\leq 10\%$.

Specificity detection

Interfering substances testing: This kit has no effect on the assay when endogenous substance including hemoglobin (200g/L), sodium heparin (3000U/L), conjugated bilirubin (86 μ M), unconjugated bilirubin (257 μ M), EDTA (7mg/mL), triglycerides (5.6mmol/L), cholesterol (6.47mmol/L), 10x Red blood cell lysis buffer, Phenol, Residual ethanol, 10x PBS, and Genomic DNA were tested.

Crossover detection: This kit detects CML or ALL-p190, CML-p230 and results show that there is no cross reaction.

Traceability

The conversion factor CF (see package label) is obtained by measuring the WHO International Standard (1st WHO International Genetic Reference Panel for the quantitation of BCR-ABL1 translocation(NIBSC code: 09/138)) at the concentration of 10%IS (MR1.0)-0.01%IS (MR4.0)using the kit, performing a linear regression between theoretical and measured values and satisfying an $R^2 > 0.97$, a slope between 0.85 and 1.10 and a 95% confidence interval for the slope of the fitted curve between 0.83 and 1.20.

Precautions

- This product is for research use only. Please read this instruction manual carefully before use.
- Before the experiment, please be familiar with the operation methods and precautions of the various instruments to be used and perform quality control for each experiment.
- The laboratory management should be strictly in accordance with the management regulations for PCR gene amplification laboratories. If cross-contamination is not well controlled during sample processing, false positive results may occur.
- Avoid repeated freezing and thawing of the reagents, mix well before use and centrifuge briefly.
- The test results are related to the quality of sample collection, processing, transportation and storage. Any mistakes in these steps can lead to inaccurate results.

Basic Information

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Date of approval and revision of this instruction manual

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