

# CBFβ-MYH11 Fusion Gene Nucleic Acid Detection Kit Digital PCR Instructions for Use

## Packaging

16 tests/kit; 48 tests/kit.

## Intended Use

This kit is intended for the quantitative detection of RNA from the CBFβ-MYH11 fusion gene and ABL1 in samples from patients with acute myeloid leukemia (AML). This kit is not intended for the diagnosis of leukemia.

## Test Principle

The kit uses specific primers and TaqMan fluorescent probes designed for the CBFβ-MYH11 fusion gene and ABL1. Complementary DNA (cDNA), reverse-transcribed from RNA extracted from patient peripheral blood, is used as the template for testing with the All-in-one Digital PCR System, enabling quantitative detection of CBFβ-MYH11. ABL1 serves as the control to monitor the extraction and testing processes.

## Kit Components

| No. | Component                 | 16 tests        | 48 tests        | Main constituents                                                                               |
|-----|---------------------------|-----------------|-----------------|-------------------------------------------------------------------------------------------------|
| 1   | Reverse Transcription Mix | 80 µL × 1 tube  | 250 µL × 1 tube | Reverse Transcriptase, RNase Inhibitor, dNTPs, Random Primers / Oligo(dT)20 VN Primer Mix, etc. |
| 2   | dPCR Reaction Mix         | 96 µL × 1 tube  | 300 µL × 1 tube | PCR Buffer, dNTPs, MgCl <sub>2</sub> , Cy5.5 Reference Dye, etc.                                |
| 3   | Primer and Probe Mix      | 16 µL × 1 tube  | 50 µL × 1 tube  | Primers and probes                                                                              |
| 4   | Positive Control          | 30 µL × 1 tube  | 30 µL × 1 tube  | cDNA mixture                                                                                    |
| 5   | Negative Control          | 30 µL × 1 tube  | 30 µL × 1 tube  | RNA from a negative cell line                                                                   |
| 6   | Nuclease-free Water       | 500 µL × 1 tube | 500 µL × 1 tube | DNase/RNase-Free Water                                                                          |

- 1) Do not mix components from kits with different lot numbers.
- 2) The kit does not include 8-tube strips. Axygen 0.2 mL 8-tube strips are recommended (catalog number: PCR-0208-C).

## Required Equipment and Reagents Not Included

Biosafety cabinet, benchtop centrifuge, vortex mixer, nuclease-free centrifuge tubes, 8-tube strips, pipette tips, nucleic acid extraction reagents, and a conventional PCR instrument or real-time quantitative PCR instrument.

## Storage Conditions and Shelf Life

Store the kit at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ . The shelf life is 12 months. Use the kit before the expiration date indicated on the kit label.

Reagents may be stored at  $2-8^{\circ}\text{C}$  for 7 days or at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 12 months. Do not exceed five freeze-thaw cycles.

## Compatible Instrument

The All-in-one Digital PCR System manufactured by Suzhou Sniper Medical Technology Co., Ltd.

## Specimen Requirements

### 1. Specimen Type

Peripheral blood.

### 2. Specimen Collection

Collect 2–10 mL of venous blood using a sterile disposable syringe and transfer the blood into a collection tube containing EDTA anticoagulant. Immediately invert the tube gently 5–10 times to mix the blood thoroughly with the anticoagulant.

### 3. Specimen Storage and Transportation

Peripheral blood samples may be processed immediately for RNA extraction and testing or stored at 2–8°C for no more than 24 h. If long-term storage of RNA samples is required, store them in a freezer at –70°C. Do not exceed two freeze-thaw cycles.

## Procedure

### 1. Nucleic Acid Extraction

1.1 The kit does not include nucleic acid extraction components. TRIzol Reagent is recommended for RNA extraction (manufacturer: Thermo Fisher Scientific (China) Co., Ltd.; catalog number: 15596018 or 15596026). Follow the extraction procedure in the reagent instructions for use.

1.2 Because RNA in peripheral blood is susceptible to degradation, RNA extraction within 24 h of collection is recommended. RNA concentration and purity may affect test results. Measuring RNA concentration and purity with a microvolume spectrophotometer is recommended, but is not mandatory. Ensure an RNA concentration >80 ng/μL and an OD<sub>260</sub>/OD<sub>280</sub> ratio >1.6. The specific requirements are as follows:

| Parameter               | Requirement                                     |
|-------------------------|-------------------------------------------------|
| Peripheral blood volume | 2–10 mL                                         |
| RNA concentration       | >80 ng/μL                                       |
| RNA purity              | OD <sub>260</sub> /OD <sub>280</sub> ratio >1.6 |

### 2. Reverse Transcription

2.1 In the reagent preparation area, remove the components required for reverse transcription and place them on ice. After the reagents have thawed completely, centrifuge briefly and return them to ice until use.

2.2 Preparation of the reverse transcription reaction: Mix the Reverse Transcription Mix thoroughly, centrifuge briefly, and dispense 5 μL into each tube of an 8-tube strip.

2.3 Sample addition: For test samples (including the Negative Control), calculate the RNA sample input volume X μL based on an RNA input of 500–1,000 ng. Add (5 – X) μL of Nuclease-free Water to obtain a final reaction volume of 10 μL. For the Negative Control, add 5 μL to a tube containing the Reverse Transcription Mix to obtain a final reaction volume of 10 μL.

Close the caps securely, vortex for 5–10 s, and centrifuge briefly. Ensure that no bubbles remain in the 8-tube strip.

2.4 Reverse transcription program:

| Step | Temperature | Time  | Cycles |
|------|-------------|-------|--------|
| 1    | 55°C        | 900 s | 1      |
| 2    | 85°C        | 300 s | 1      |

2.5 Perform reverse transcription using a conventional PCR instrument or real-time quantitative PCR instrument.

### 3. Amplification and Detection

3.1 In the reagent preparation area, remove the kit components and place them on ice. After the reagents have thawed completely, centrifuge briefly and return them to ice until use.

**3.2 Preparation of the reaction mix:** Calculate the required volume of each component according to the number of Positive Controls, Negative Controls, and test samples. The kit is designed for a final reaction volume of 22  $\mu$ L. Prepare the mix for each reaction as follows:

| Reaction component   | Volume per reaction |
|----------------------|---------------------|
| dPCR Reaction Mix    | 6 $\mu$ L           |
| Primer and Probe Mix | 1 $\mu$ L           |

Mix the above components thoroughly, centrifuge briefly, and dispense 7  $\mu$ L into each tube of an 8-tube strip.

### 3.3 Sample addition:

**Controls:** Add 5  $\mu$ L of the Positive Control and 5  $\mu$ L of the Negative Control cDNA solution to separate tubes. Add Nuclease-free Water to obtain a final reaction volume of 22  $\mu$ L in each tube.

**Test samples:** Calculate the reverse-transcribed cDNA sample input volume Y  $\mu$ L based on an input of 300–500 ng. Add Nuclease-free Water to obtain a final reaction volume of 22  $\mu$ L.

Close the caps securely, vortex for 5–10 s, and centrifuge briefly. Ensure that no bubbles remain in the 8-tube strip.

**Note 1:** A Negative Control and a Positive Control must be included in each test run.

**Note 2:** Ensure that the number of reactions in each run is a multiple of four (i.e., 4, 8, 12, or 16 reactions). Fill any unused reaction positions with 22  $\mu$ L of Nuclease-free Water.

## 4. Sample Setup and Amplification Program

**4.1** Load the required consumables in accordance with the User Manual for the All-in-one Digital PCR System.

**4.2** Click “Method” and set the following amplification program:

| Step | Stage                   | Temperature | Time  | Cycles |
|------|-------------------------|-------------|-------|--------|
| 1    | Droplet stabilization   | 60°C        | 300 s | 1      |
| 2    | Enzyme activation       | 95°C        | 900 s | 1      |
| 3    | Denaturation            | 95°C        | 10 s  | 45     |
| 4    | Annealing and extension | 60°C        | 30 s  |        |

Fluorescence channels: CBF $\beta$ -MYH11, VIC; ABL1, ROX; droplet identification, FAM.

**Note:** Set the heating ramp rate to 2°C/s throughout the amplification program.

**4.3** Fluorescence channel settings: Select “VIC” for CBF $\beta$ -MYH11, “ROX” for ABL1, and “FAM” for droplet identification.

## 5. Result Analysis

### 5.1 Viewing Results and Setting Thresholds

- After the dPCR run is completed, open “Analysis Plot” → “1D Scatter Plot” or “2D Scatter Plot” → “Plate Map” to view the amplification results for each well.
- Select the Positive Control well and choose “All”. Set the threshold at one-third of the distance between the negative and positive droplet clusters, closer to the negative cluster, and click “Apply”.
- Check all reaction wells to ensure that the threshold does not pass through any droplet cluster.
- If the threshold does not adequately separate the negative and positive droplet clusters in a test sample, or passes through a cluster, set an individual threshold for that sample as described in step b (select “Single”).
- The number of positive droplets, concentration, and other results are obtained based on the thresholds set.

### 5.2 Exporting Results

Insert a USB flash drive into the instrument USB port. Open the “Analysis” menu in the software and click “Export” to export the results of the current run.

### 5.3 Result Calculation

$$\text{Copy number (copies)} = \frac{C \times N \times 0.6}{1,000}$$

C = concentration measured by the instrument (copies/μL); N = number of accepted droplets ("Nb Droplets"); droplet volume = 0.6 nL.

$$\text{Ratio (\%)} = \frac{\text{CBF}\beta\text{-MYH11 copy number}}{\text{ABL1 copy number}} \times 100\%$$

### 6. Quality Control

6.1 The Negative Control and Positive Control must meet the following requirements. If these requirements are not met, repeat the test run.

| Control          | Expected result                                                     |
|------------------|---------------------------------------------------------------------|
| Positive Control | CBFβ-MYH11 Ratio: 10%; acceptable relative deviation: ±30% (7%–13%) |
| Negative Control | CBFβ-MYH11 copy number <1                                           |

6.2 Droplet count: Each reaction well must contain at least 18,000 accepted droplets. If this requirement is not met, repeat testing for the affected well.

6.3 If all the above requirements are met, the results are valid and may be analyzed further. If the requirements in Section 6.1 are met but some wells do not meet Section 6.2, repeat testing only for the wells that fail Section 6.2.

### Positive Cutoff

When the kit quality control requirements are met, the result is positive if the number of CBFβ-MYH11-positive droplets is ≥1. When the kit quality control requirements are met, the result is negative if the number of CBFβ-MYH11-positive droplets is <1.

### Interpretation of Results

1. Provided that the quality control requirements in Sections 6.1–6.3 are met, interpret the results as follows:

| Test result                 | Reported result                                | Interpretation                                                                                                                      |
|-----------------------------|------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|
| CBFβ-MYH11 copy number = 0  | Negative                                       | Indicates that the sample contains no CBFβ-MYH11 fusion gene.                                                                       |
| Ratio (%) <0.002%           | Fusion gene detected, but cannot be quantified | The result is below the kit limit of detection and must not be used as a basis for judgment.                                        |
| 0.002% ≤ Ratio (%) <0.0032% | Fusion gene detected, but cannot be quantified | CBFβ-MYH11 fusion gene detected. The result is for reference only. These samples may yield no numerical result upon repeat testing. |
| Ratio (%) ≥0.0032%          | Fusion gene detected; report Ratio (%)         | CBFβ-MYH11 fusion gene detected.                                                                                                    |

Notes:

- (1) The copy number linear range of the compatible instrument (the All-in-one Digital PCR System) is 3–150,000 copies. Results within the linear range are recommended for analysis.
- (2) If the ABL1 copy number is ≤32,000 and the CBFβ-MYH11 copy number is 0, the ABL1 copy number is too low. Repeat testing with an increased sample input may be performed.
- (3) If the ABL1 copy number is >150,000 and the CBFβ-MYH11 copy number is >0, the result may be reported. However, the ABL1 copy number exceeds the linear range and may affect quantitative accuracy. Repeat testing with a reduced sample input may be performed.

### Performance Characteristics

| Characteristic | Specification                                                                        |
|----------------|--------------------------------------------------------------------------------------|
| Agreement      | Testing of in-house negative and positive reference materials yields 100% agreement. |

| Characteristic          | Specification                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|-------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Limit of blank          | All five tests of the in-house limit-of-blank reference material are negative (100%).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Limit of detection      | All five tests of the in-house limit-of-detection reference material (Ratio: 0.002%) are positive.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Limit of quantification | All five tests of the in-house limit-of-quantification reference material (Ratio: 0.0032%) are positive. The absolute value of the coefficient of variation of the logarithms of the measured Ratio (%) values is $\leq 10\%$ .                                                                                                                                                                                                                                                                                                                                                   |
| Precision               | In five tests of the in-house precision reference material, the coefficient of variation of the measured Ratio (%) values is $\leq 10\%$ .                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Specificity             | <p>Interference: The following substances have no effect on detection at the tested concentrations: hemoglobin (<math>\leq 100</math> g/L), heparin sodium (<math>\leq 24</math> mg/L), total bilirubin (<math>\leq 257</math> <math>\mu</math>M), EDTA (<math>\leq 5</math> mg/mL), triglycerides (<math>\leq 5.6</math> mM), and cholesterol (<math>\leq 6.47</math> mM).</p> <p>Cross-reactivity: No cross-reactivity is observed when testing CML-P210, CML-P230, CML or ALL-P190, PML/RARA, AML1-ETO, TEL/AML1, E2A/PBX1, MLLT3/MLL, MLL/AF4 (e9e5), or MLL/AF4 (e10e4).</p> |

### Precautions

1. For Research Use Only. Read these instructions carefully before use.
2. Before testing, become familiar with the operating procedures and precautions for all instruments to be used. Perform quality control for every test run.
3. Laboratory management must strictly follow the requirements for PCR gene amplification laboratories. Failure to control cross-contamination during sample processing may cause false-positive results.
4. Avoid repeated freezing and thawing of reagents. Mix thoroughly and centrifuge briefly before use.
5. Test results depend on the quality of sample collection, processing, transportation, and storage. Errors at any of these stages may lead to inaccurate results.

### Manufacturer Information

Company: Suzhou Sniper Medical Technology Co., Ltd.

Manufacturing address: Rooms 301 and 401, Building 15, No. 52 Yingang Road, Fuqiao Town, Taicang, Jiangsu, China.

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### Instructions for Use Approval and Revision Dates

Approval date: September 15, 2025.