

## Specification

16/48 tests/kit

## Product Description

This kit is intended for the qualitative detection of mutations in the human isocitrate dehydrogenase 1 (IDH1) gene in DNA extracted from peripheral blood samples of individuals with acute myeloid leukemia (AML), for research purposes only. This product is for research use only. Not for use in diagnostic procedures.

The IDH1 gene is located on chromosome 2q33.3 and contains 10 exons. The IDH1 protein encoded by this gene is found in the cytoplasm and peroxisomes, where it functions as a key catalytic enzyme in the tricarboxylic acid (TCA) cycle. Its primary role is to catalyze the oxidative decarboxylation of isocitrate to  $\alpha$ -ketoglutarate ( $\alpha$ -KG) in the cytoplasm. This process contributes to cellular energy metabolism and serves as a major source of cytosolic NADPH, which is essential for various biosynthetic and redox reactions.

Recent studies have shown that the mutation frequency of the IDH1 gene varies among different AML populations. In adults, the reported frequency of IDH1 mutations ranges from 4.3% to 33%, while in pediatric populations, it ranges from 1.3% to 1.7%.

This kit is intended solely for non-clinical laboratory research use. It is not intended for the diagnosis, monitoring, or treatment of acute myeloid leukemia or any other disease. Results obtained with this kit are for research reference only and should not be used as the sole basis for medical or clinical decision-making. Researchers are advised to consider additional molecular, phenotypic, and contextual data when interpreting results.

### DNA Detection Locus Information:

| Chromosome | Gene Detected | Exon | Amino Acid Mutation                                  |
|------------|---------------|------|------------------------------------------------------|
| Chr2       | IDH1          | 2    | p.R132H, p.R132C, p.R132G, p.R132S, p.R132L, p.R132V |

This kit targets the IDH1 gene R132 mutation hotspot region (without distinguishing specific mutation subtypes) for analysis. It employs a drop-off probe design strategy, using specific primers to amplify the target region via PCR. The detection is performed using DQ24 Series Digital PCR System, with the VIC and ROX fluorescence channels used to distinguish wild-type and mutated sequences. DNA extracted from peripheral blood samples is used as the template, enabling the detection of mutations at the R132 site of the IDH1 gene.

## Contents and Specification

| No. | Content                | Amount      |             | Main Component                                                                                   |
|-----|------------------------|-------------|-------------|--------------------------------------------------------------------------------------------------|
|     |                        | 16 tests    | 48 tests    |                                                                                                  |
| 1   | dPCR Master Mix        | 110 $\mu$ L | 300 $\mu$ L | 4 $\times$ DNA Probe Buffer, dNTP Mix, MgCl <sub>2</sub> , FAM Reference Dye, Taq DNA Polymerase |
| 2   | Primer Probe Mix       | 16 $\mu$ L  | 50 $\mu$ L  | Primers, Probe                                                                                   |
| 3   | Positive Control       | 15 $\mu$ L  | 15 $\mu$ L  | Plasmid DNA Mix                                                                                  |
| 4   | Negative Control       | 15 $\mu$ L  | 15 $\mu$ L  | Wild type DNA                                                                                    |
| 5   | DNase/RNase-Free Water | 500 $\mu$ L | 500 $\mu$ L | 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  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$  with a shelf life of 12 months. Use within the expiration date indicated on the kit.
- Reagents stored at  $2-8^{\circ}\text{C}$  are stable for 7 days; stored at  $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ , they are stable for 12 months. However, they should not be subjected to more than 5 freeze-thaw cycles.

## 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 DNA extraction and detection immediately or stored at  $4^{\circ}\text{C}$  for no more than 24 hours.
  - b) For long-term storage, extracted and purified DNA should be stored at  $-20 \pm 5^{\circ}\text{C}$  for no more than 3 months, with  $\leq 5$  allowable freeze-thaw cycles.
  - c) For transportation, use dry ice and ensure transit time does not exceed 110 hours.

## Methods

### 1. Nucleic Acid Extraction

1. This kit does not contain nucleic acid extraction components. Select an appropriate whole blood DNA extraction kit based on the sample type. Perform extraction according to the instructions provided in the kit manual.
2. Use the recommended DNA quantification kit (e.g., Qubit dsDNA HS Assay, Thermo Fisher Scientific, Cat. No. Q32851) to measure the concentration of extracted DNA. A DNA input amount  $>20$  ng (with a maximum loading volume of  $10\ \mu\text{L}$ ) is considered acceptable.

### 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  $22\ \mu\text{L}$ . Prepare the reaction solution as follows:

| Reaction Component | Volume           |
|--------------------|------------------|
| dPCR Master Mix    | $6\ \mu\text{L}$ |
| Primer Probe Mix   | $1\ \mu\text{L}$ |

After thoroughly mixing the above reaction solution, briefly centrifuge it and dispense it into 8-tube strips at  $7\ \mu\text{L}$  per tube.

3. Sample addition
  - i. **Control samples:** Take  $2\ \mu\text{L}$  each of the positive and negative controls and add them to separate 8-tube strips respectively. Then add  $13\ \mu\text{L}$  of nuclease-free water to make up a final reaction system of  $22\ \mu\text{L}$ .
  - ii. **Samples to be tested:** Calculate the volume  $X\ \mu\text{L}$  of the DNA sample to be added. Then add  $(15-X)\ \mu\text{L}$  of nuclease-free water to make up a final reaction system of  $22\ \mu\text{L}$ .
  - 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 | Droplet spreading     | 60°C        | 300s | 1     |
| 2 | Enzyme activation     | 95°C        | 300s | 1     |
| 3 | Denaturation          | 95°C        | 10s  | 45    |
| 4 | Annealing & Extension | 63°C        | 30s  |       |

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

3. Fluorescence channel selection:

| Channel                          | VIC                | ROX       |
|----------------------------------|--------------------|-----------|
| Target                           | Internal Reference | Wild Type |
| 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".

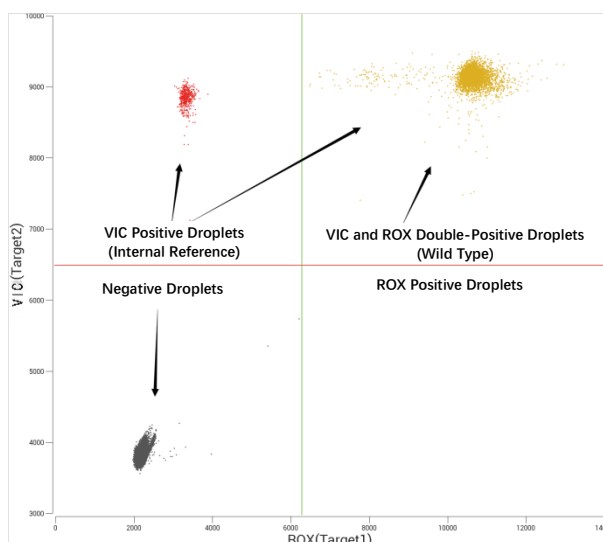


Figure 1 Positive IDH1 mutation 2D Scatter Plot

- 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 Interpretation:
 

Droplets that are positive for ROX only should be excluded from analysis.
3. 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.

4. Result calculation:  
The kit reports results as mutation frequency, calculated as:

$$\text{Mutation Frequency (\%)} = \frac{\text{Mutation Copy Number}}{\text{Internal Reference Copy Number}} \times 100\%$$

$$\text{R132 Mutation Frequency (\%)} = \frac{\text{VIC} - \text{ROX}}{\text{VIC}} \times 100\%$$

Note:

Mutation frequency can be calculated using either the instrument detection values or copy numbers.

Nb Droplet: Valid droplet count.

C: Instrument detection value (copy/ $\mu$ L).

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

$$\begin{aligned} \text{Mutation Copy Number} \\ &= \text{Internal Copy Number (VIC Positive Copy Number)} \\ &\quad - \text{Wild Type Copy Number (ROX Positive Copy Number)} \end{aligned}$$

## 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 | Positive Control Mutation Frequency=10% (acceptable range: $\pm 30\%$ of target value) |
| Negative Control | VIC single-positive droplet 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 2D dot plot result is considered positive when the number of VIC single-positive droplets  $\geq 1$ . If the VIC single-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                                                                                                                     |
|---------------------------------|---------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| Mutant copy number = 0          | Negative      | No IDH1 mutation detected in the sample.                                                                                                  |
| Mutation frequency < 0.1%       | Positive      | The result exceeds the lower limit of detection of the kit and result is for reference only. Repeated testing may yield negative results. |
| Mutation frequency $\geq 0.1\%$ | Positive      | IDH1 mutation detected.                                                                                                                   |

Note:

- A mutation frequency of 0.1% is achievable with an input of 20 ng of DNA. Increasing the input amount within the acceptable range may enhance detection sensitivity.
- When the reference gene signal (measured in cp/ $\mu$ L by the instrument) is less than 60:

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Please visit [www.sniper-tech.com](http://www.sniper-tech.com) for a complete listing of recommended products.

If the result is negative, it is recommended to increase the DNA input and repeat the test.  
If the result is positive, it may be reported as IDH1 mutation positive.

## Product Performance Specifications

|                          |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Concordance Rate         | <p>Negative Reference Material Concordance:<br/>The kit tested enterprise negative reference materials once each, achieving 100% negative concordance.</p> <p>Positive Reference Material Concordance:<br/>The kit tested enterprise positive reference materials once each, achieving 100% positive concordance.</p>                                                                                                                                                                                                                                                                                                               |
| Limit of Detection (LoD) | The kit was tested with an enterprise-provided reference material containing an IDH1 mutation frequency of 0.1% at a DNA input of 20 ng. All 10 replicates yielded positive results, confirming the detection limit at this mutation frequency.                                                                                                                                                                                                                                                                                                                                                                                     |
| Precision                | The kit tested standardized enterprise precision reference materials 10 times, with 100% positive results and a CV $\leq 10\%$ for measured values of 10% reference materials.                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Specificity              | <p>Interference Resistance Testing<br/>The kit was tested against endogenous interferents at the following concentrations, with no impact on detection: Hemoglobin (<math>\leq 2</math> mg/L), Bilirubin (350 <math>\mu\text{M/L}</math>), EDTA (<math>\leq 2</math> mg/mL), Triglycerides (<math>\leq 37</math> mmol/L), Cholesterol (<math>\leq 6.47</math> mM), Ethanol (<math>\leq 21.7</math> mmol/L).</p> <p>Cross-reactivity Testing<br/>No cross-reactivity was observed with other AML-related mutations. The assay can tolerate up to 200 ng of wild-type human genomic DNA without producing false-positive results.</p> |

## 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:

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