

Specification

16/48 tests/kit

Product Description

This kit is intended for the qualitative detection of mutations in the human isocitrate dehydrogenase 2 (IDH2) gene in DNA extracted from peripheral blood samples of individuals with acute myeloid leukemia (AML), for research use only.

This product is for research use only. Not for use in diagnostic procedures.

The IDH2 gene encodes isocitrate dehydrogenase 2 (IDH2), a key enzyme localized in the mitochondria. IDH2 plays a critical role in cellular metabolism by catalyzing the conversion of isocitrate to α -ketoglutarate (α -KG) and generating reduced nicotinamide adenine dinucleotide phosphate (NADPH).

α -KG is a vital metabolic intermediate involved in amino acid metabolism, cellular energy balance, DNA repair, epigenetic regulation, and antioxidant defense. NADPH serves as a reducing agent for biosynthetic processes such as fatty acid and steroid synthesis, as well as oxidative stress mitigation.

Mutations in the IDH2 gene are frequently observed in various tumor types, particularly at hotspot codons R140 and R172. These mutations result in a gain-of-function enzyme that catalyzes the aberrant conversion of α -KG to 2-hydroxyglutarate (2-HG). 2-HG is considered an oncometabolite that can inhibit multiple α -KG-dependent enzymes, including histone demethylases and DNA demethylases, thereby disrupting normal epigenetic regulation.

This kit is intended solely for non-clinical research applications. It is not intended for the diagnosis, monitoring, or treatment of any disease. Results obtained with this kit are for research reference only and should not be used as the sole basis for any decision-making. Researchers should interpret results in the context of broader experimental findings.

DNA Detection Locus Information:

Chromosome	Gene Detected	Exon	Amino Acid Mutation
Chr15	IDH2	4	p.R140W, p.R140L, p.R140Q, p.R172K, p.R172M, p.R172G, p.R172S, p.R172.W

This kit targets the R140 and R172 mutation hotspot regions of the IDH2 gene (without distinguishing specific mutation subtypes). It adopts a drop-off assay design, in which specific primers are used to amplify the target regions by PCR. Mutation detection is achieved using probes labeled with VIC, CY5, and ROX to distinguish allelic variants.

Using DNA extracted from peripheral blood samples, the assay is performed on DQ24 Series All-in-one Digital PCR system, enabling the qualitative detection of mutations in the IDH2 R140 and R172 regions.

Contents and Specification

No.	Content	Amount		Main Component
		16 tests	48 tests	
1	dPCR Master Mix	110 μ L	300 μ L	4 $\times$ DNA Probe Buffer, dNTP Mix, MgCl ₂ , FAM Reference Dye, Taq DNA Polymerase
2	Primer Probe Mix	16 μ L	50 μ L	Primers, Probe
3	Positive Control	15 μ L	15 μ L	Plasmid DNA Mix
4	Negative Control	15 μ L	15 μ L	Wild type DNA
5	DNase/RNase-Free Water	500 μ L	500 μ 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.

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Please visit www.sniper-tech.com for a complete listing of recommended products.

3. 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°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 ≤ 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 $15\ \mu\text{L}$) 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	900s	1
3	Denaturation	95°C	10s	45
4	Annealing & Extension	60°C	30s	

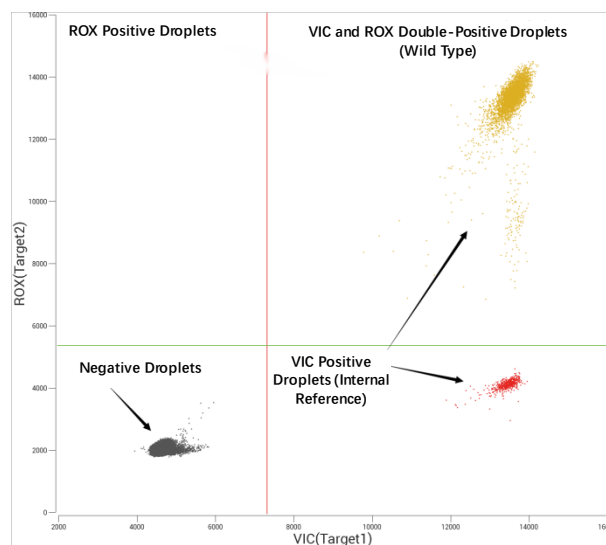
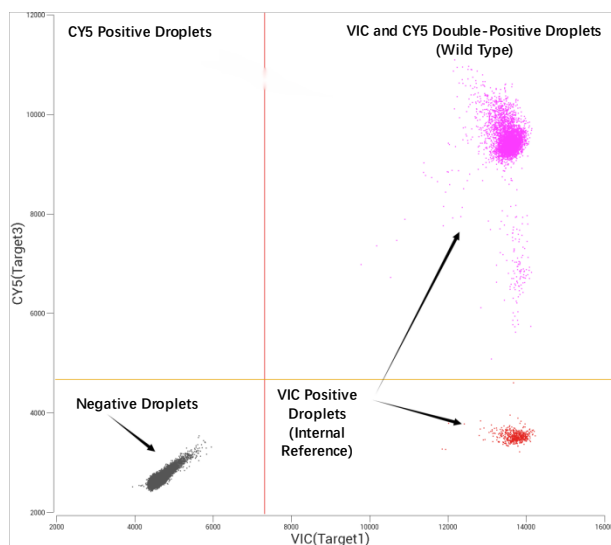
Note: The temperature ramp rate should be set to 2°C/s throughout the entire amplification program.

3. Fluorescence channel selection:

Channel	VIC	CY5	ROX
Target	Internal Reference	R140 Wild Type	R172 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".



- 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 and CY5 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:

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

$$\text{R140 Mutation Frequency (\%)} = \frac{\text{VIC} - \text{CY5}}{\text{VIC}} \times 100\%$$

$$\text{R172 Mutation Frequency (\%)} = \frac{\text{VIC} - \text{ROX}}{\text{VIC}} \times 100\%$$

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/μL).

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

$$\begin{aligned} \text{R172 Mutation Copy Number} \\ &= \text{Internal Copy Number (VIC Positive Copy Number)} \\ &\quad - \text{Wild Type Copy Number (ROX Positive Copy Number)} \end{aligned}$$

$$\begin{aligned} \text{R140 Mutation Copy Number} \\ &= \text{Internal Copy Number (VIC Positive Copy Number)} \\ &\quad - \text{Wild Type Copy Number (CY5 Positive Copy Number)} \end{aligned}$$

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: ±30% of target value)
Negative Control	VIC single-positive droplet number < 2

2. Valid droplet count requirement:
Each reaction well must generate ≥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 single-positive droplet ≥2. If the single-positive droplet count < 2 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 IDH2 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	IDH2 mutation detected.

Note:

1. 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.
2. When the reference gene signal (measured in cp/μL by the instrument) is less than 60:
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 IDH2 mutation positive.

Product Performance Specifications

Concordance Rate	Negative Reference Material Concordance: The kit tested enterprise negative reference materials once each, achieving 100% negative concordance. Positive Reference Material Concordance: The kit tested enterprise positive reference materials once each, achieving 100% positive concordance.
Limit of Detection (LoD)	The kit was tested with an enterprise-provided reference material containing an IDH2 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	Interference Resistance Testing The kit was tested against endogenous interferents at the following concentrations, with no impact on detection: Hemoglobin ($\leq$ 2 mg/L), Bilirubin (350 μM/L), EDTA ($\leq$ 2 mg/mL), Triglycerides ($\leq$ 37 mmol/L), Cholesterol ($\leq$ 6.47 mM), Ethanol ($\leq$ 21.7 mmol/L). Cross-reactivity Testing 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.

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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