

Specification

16/48 tests/kit

Product Description

This kit is designed for the detection of human KIT gene D816H mutations. For research use only.

DNA Detection Locus Information:

Chromosome	Gene Detected	Exon	Amino Acid Mutation	Nucleotide Mutation	Cosmic ID
Chr4	KIT	17	p.D816H	c.2446G>C	1311

The kit targets the KIT gene D816H mutation allele region using specific primers for PCR amplification. Probes recognizing mutant and wild-type are labeled with VIC and ROX, respectively. Using DNA extracted from patient peripheral blood as a template, the All-in-one Digital PCR Instrument detects and quantifies mutant copy number and mutation frequency.

Contents and Specification

No.	Content	Amount		Main Component
		16 tests	48 tests	
1	dPCR Master Mix	176µL	550µL	2× DNA Probe Buffer, dNTP Mix, MgCl ₂ , FAM Reference Dye, Taq DNA Polymerase
2	Primer Probe Mix	16µL	50µL	Primers, Probe
3	Positive Control	15µL	15µL	Plasmid DNA Mix
4	Negative Control	15µL	15µL	Wild type DNA
5	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.

Compatible Instrument

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

Sample Requirements

- Applicable Sample Type: Peripheral blood samples.
- 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.
- Sample Storage and Transportation

Suzhou Sniper Medical Technologies Co., Ltd.

E-mail: sniper@sniper-tech.com

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

- Peripheral blood samples can be processed for DNA extraction and detection immediately or stored at 4°C for no more than 24 hours.
- 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.
- For transportation, use dry ice and ensure transit time does not exceed 110 hours.

Methods

1. Nucleic Acid Extraction

- 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.
- 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 μL) is considered acceptable.

2. Reaction Mix

- 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.
- 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 μL . Prepare the reaction solution as follows:

Reaction Component	Volume
dPCR Master Mix	11 μL
Primer Probe Mix	1 μL

After thoroughly mixing the above reaction solution, briefly centrifuge it and dispense it into 8-tube strips at 12 μL per tube.

- Sample addition
 - Control samples:** Take 2 μL each of the positive and negative controls and add them to separate 8-tube strips respectively. Then add 8 μL of nuclease-free water to make up a final reaction system of 22 μL .
 - Samples to be tested:** Calculate the volume X μL of the DNA sample to be added according to an amount of 20ng. Then add $(10-X)$ μL of nuclease-free water to make up a final reaction system of 22 μL .
 - 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:

- A negative control and a positive control should be set up for each test.
- 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 22 μL of nuclease-free water.

3. Program Setup

- Place the corresponding consumables according to the *Sniper All-in-one Digital PCR Instrument User Manual*.
- 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	60°C	30s	

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

- Fluorescence channel selection:
D816H wild type: ROX
D816H mutant: VIC

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

$$\text{Mutation Frequency (\%)} = \frac{\text{Mutant}}{\text{Mutant} + \text{Wild Type}} \times 100\% = \frac{\text{VIC}}{\text{VIC} + \text{ROX}} \times 100\%$$

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).

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

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	Mutant single-positive droplet number < 1
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 result is considered positive when the number of single-positive droplet ≥1. If the single-positive droplet 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 D816H mutation detected in the sample.
Mutation frequency < 0.1%	Positive	Detected but below LoD; interpret with caution / for reference only; retest recommended.

Mutation frequency $\geq 0.1\%$ Positive D816H mutation detected.

Note: If the combined instrument detection value (wild-type + mutant, cp/ μ L) < 60 OR the total copy number (cp) < 1000 :

1. Negative results: Increase the sample volume and repeat testing.
2. Positive results: Report the corresponding mutation as 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 Blank (LoB)	Test enterprise LoB reference materials 10 times. 100% negative results.
Limit of Detection (LoD)	The kit tested single wells of enterprise LoD reference materials (standardized to 0.1% mutation frequency) 10 times, with 100% positive results and a CV $\leq 10\%$ for measured values.
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 (≤ 2 mg/mL), Bilirubin (350 μM/L), EDTA (≤ 2 mg/mL), Triglycerides (≤ 37 mmol/L), Cholesterol (≤ 6.47 mM), Ethanol (21.7 mmol/L). Cross-reactivity Testing No cross-reactivity was observed with other KIT genes. The kit can tolerate 200 ng of wild type human genomic DNA.

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

Company Name: Suzhou Sniper Medical Technology Co., Ltd.

Manufacturing Address: Room 301 & 401, Building 15, No. 52 Yingang Road, Fuqiao Town, Taicang City, Jiangsu Province, China.

Contact Information: +86-512-62809514

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Suzhou Sniper Medical Technologies Co., Ltd.

E-mail: sniper@sniper-tech.com

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