

Specification

16/48 tests/kit

Product Description

This kit is intended for the qualitative detection of the L265P mutation in the human MYD88 gene using DNA extracted from peripheral blood samples.

For Research Use Only. Not for use in diagnostic procedures.

The MYD88 (myeloid differentiation primary response 88) gene encodes an adaptor protein that mediates signaling from Toll-like receptors (TLRs) and interleukin-1 receptors (IL-1Rs). The c.794T>C (L265P) point mutation in MYD88 is the most frequently observed variant, leading to constitutive activation of downstream NF-κB signaling, which promotes sustained proliferation of tumor cells.

The MYD88 L265P mutation is present in approximately 90% of lymphoplasmacytic lymphoma/Waldenström's macroglobulinemia (LPL/WM) cases and 60–80% of IgM-type monoclonal gammopathy of undetermined significance (MGUS) cases.

This kit is not intended for the diagnosis of lymphoid malignancies. Results obtained are for research reference only and should not be used as the sole basis for disease evaluation. Clinical interpretation should be based on a comprehensive assessment of the subject's clinical presentation and additional laboratory findings.

DNA Detection Locus Information:

Chromosome	Gene Detected	Exon	Amino Acid Mutation	Nucleotide Mutation	Cosmic ID
Chr3	MYD88	5	p.L265P	c.794T>C	85940

This kit targets the L265P mutation region of the MYD88 gene. Specific primers are used to amplify the target sequence by PCR.

Mutation detection is achieved using allele-specific probes labeled with VIC (for the mutant allele) and ROX (for the wild-type allele).

DNA extracted from peripheral blood is used as the template. Detection is performed using a digital PCR system, enabling sensitive and accurate assessment of the MYD88 L265P mutation status.

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.

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

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 $10 \mu\text{L}$) 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	$11 \mu\text{L}$
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 $12 \mu\text{L}$ 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 $8 \mu\text{L}$ 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 $(10-X) \mu\text{L}$ 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	

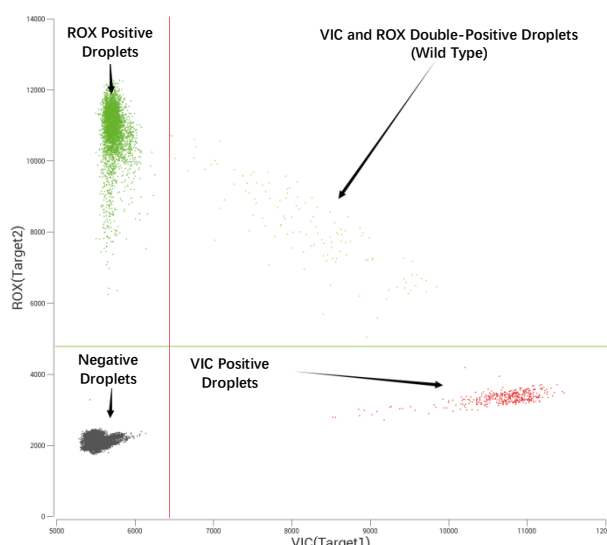
Note: The temperature ramp rate should be set to 2°C/s throughout the entire amplification program.

3. Fluorescence channel selection:

Channel	ROX	VIC
Target	L265P Wild Type	L265P Mutation
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 L265P mutation detected in the sample.
Mutation frequency < 0.1%	Positive	L265P mutation is detected but the measured value is below the kit's sensitivity threshold, the result should not be used as a basis for interpretation. To improve detection sensitivity, it is recommended to increase the DNA input amount within the acceptable range.
Mutation frequency ≥ 0.1%	Positive	L265P mutation detected.

Note:

1. A 0.1% mutation frequency is detectable with a 20 ng DNA input. Increasing the input amount within the tolerance range may further enhance assay sensitivity.
2. When the combined signal of wild-type and mutant alleles is less than 60 cp/μL, or the total copy number is below 1,000 copies, the following recommendations apply:
If the result is negative, it is advisable to increase the DNA input and repeat the test.
If the result is positive, it may be considered reportable as a valid mutation detection result.

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 tested single wells of enterprise LoD reference materials (standardized to 0.1% mutation frequency) 10 times, with 100% positive results.
Precision	The kit was tested using an enterprise-provided precision reference material in five replicates. All results were positive, with a coefficient of variation (CV) of the log-transformed mutation frequency values $\leq 10\%$.
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 genes commonly mutated in hematologic disorders, such as KIT mutations. The assay can tolerate up to 200 ng of wild-type human genomic DNA without producing false positives.

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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