

Specification

48 tests/kit

Product Description

This kit is used for qualitative detection of BRAF, TERT, TP53 and RET gene mutations in thyroid nodule samples that cannot be determined as benign or malignant. This product is intended for research use only in thyroid cancer cases within Bethesda Report Class III and IV populations, where fine needle aspiration biopsy cannot determine malignancy.

BRAF gene V600E, V600K, K601E, TERT gene C228T, C250T, TP53 gene R273H, and RET gene M918T mutations are used as target sequences. Specific primers are used for high-precision PCR amplification of target sequences. Different mutants and wild-type probes are set up. DNA from fine needle aspiration biopsy is used as a template for detection by digital PCR machine, thus achieving the detection of mutant copy number and mutation frequency. The kit uses wild type as a control to monitor the extraction and detection process.

DNA Detection Locus Information:

Gene Detected	Amino Acid Mutation	Nucleotide Mutation
BRAF	p.V600E	c.1799T>A
	p.K601E	c.1801A>G
	p.V600E	c.1799_1800delinsAA
	p.V600K	c.1798_1799delinsAA
RET	p.M918T	c.2753T>C
TP53	p.R273H	c.818G>A
TERT	/	C250T
	/	C228T

Contents and Specification

No.	Content	Amount	Main Component
1	dPCR Master Mix	1000μL	Hot Start Taq DNA Polymerases, dPCR Buffer, dNTPs, MgCl ₂ , Cy5.5 Reference Dye
2	Primer Probe Mix Tube 1	50μL	Primer, Probe (BRAF V600E(c.1799T>A), TERT C228T, TERT C250T, TP53 R273H, internal control)
3	Primer Probe Mix Tube 2	50μL	Primer and Probe (BRAF V600E(c.1799_1800delinsAA), V600K, K601E, RET M918T, internal control)
4	Positive Control Tube 1	30μL	Plasmid DNA mix
	Positive Control Tube 2	30μL	Plasmid DNA mix
5	Negative Control	30μL	Wild type DNA
6	DNase/RNase-Free Water	500μL	DNase/RNase-Free Water

Note:

1. The components in different lots of kits cannot be mixed.
2. The kit does not contain 8-tube strips. Axygen 0.2mL 8-tube strips (Cat. No. PCR-0208-C) are recommended.
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 $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ are stable for 12 months. Reagents should not be repeatedly frozen and thawed more than 5 times.

Compatible Instrument

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

Sample Requirements

1. Applicable sample types: formalin-fixed and paraffin-embedded tumor tissue samples (FFPE) or thyroid nodule fine needle aspiration biopsy samples.
2. Sample collection: After the collection of thyroid nodule fine needle aspiration biopsy samples, they should be stored in the sample preservation solution as soon as possible, using Sniper sample preservation solution at -15°C or below. The sample preservation time should not exceed 6 months, and the sample should be repeatedly frozen and thawed no more than 4 times.

Tumor tissue FFPE samples: Confirmed by pathological evaluation to have a tumor cell content of not less than 10%, should be stored for no more than 3 years, with a slice thickness between 5-10 μm and a minimum of 5 slices.

The FFPE sample was extracted using the FFPE sample nucleic acid extraction kit from Suzhou Sniper Medical Technology Co., Ltd. The extracted nucleic acid was measured for concentration using a UV spectrophotometer, and the DNA concentration was not less than $2\text{ng}/\mu\text{L}$. It is recommended to test the extracted DNA immediately, otherwise please store it at $-20 \pm 5^{\circ}\text{C}$ for no more than 6 months.

Methods**1. Nucleic Acid Extraction**

1. According to the sample type, select the appropriate nucleic acid extraction kit: tissue (FFPE) extraction is recommended to use FFPE extraction kit from Suzhou Sniper medical technology Co., Ltd. Use the nucleic acid extraction kit (also produced by Suzhou Sniper medical technology Co., Ltd.) to extract nucleic acid from thyroid nodule fine needle aspiration biopsy samples. Extract according to the corresponding instructions.
2. DNA quantification: measure nucleic acid concentration using a UV spectrophotometer. Sample of each reaction well shall not be less than 20ng.

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 μ L. Prepare the reaction solution as follows:

Reaction Component	Volume
dPCR Master Mix	10 μ L
Primer Probe Mix Tube1/2	1 μ L
Sample	1-11 μ L ($\geq$ 20ng)
DNase/RNase-Free Water	Up to 22 μ L
Total	22 μ L

3. Sample addition

- Control samples:** Take 5 μ L each of the positive and negative controls and add them to separate 8-tube strips respectively. Then add nuclease-free water to make up a final reaction system of 22 μ L.
- Sample to be tested:** Calculate and add the volume of DNA sample (X μ L) based on the mass of $\geq$ 20ng and add nuclease-free water to make up a final reaction system of 22 μ L.
- 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 μ L 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	900s	1
3	Denaturation	95°C	30s	45
4	Annealing & Extension	62°C	45s	

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

3. Fluorescence channel selection:

No.	Channel	FAM	HEX	ROX	CY5	ATTO425
Tube 1	Target	TERT C228T	TERT C250T	TP53 R273H	BRAF V600E (c.1799T>A)	Internal control
Tube 2		BRAF K601E	BRAF V600E (c.1799_1800delinsAA)	BRAF V600K	RET M918T	Internal control

Droplet recognition channel: CY5.5

4. Result Analysis

- 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:
Tube 1:

$$TERT\ C228T\ Mutation\ Frequency\ (\%) = \frac{FAM}{ROX + ATTO425} \times 100\%$$

$$TERT\ C250T\ Mutation\ Frequency\ (\%) = \frac{HEX}{ROX + ATTO425} \times 100\%$$

$$TP53\ R273H\ Mutation\ Frequency\ (\%) = \frac{ROX}{ROX + ATTO425} \times 100\%$$

$$BRAF\ V600E(c.1799T > A)\ Mutation\ Frequency\ (\%) = \frac{CY5}{ROX + ATTO425} \times 100\%$$

Tube 2:

$$BRAF\ V600E(c.1799_1800delinsAA)\ Mutation\ Frequency\ (\%) = \frac{HEX \times (1 - TP53\ R273H\ Mutation\ Frequency)}{ATTO425} \times 100\%$$

$$BRAF\ K601E\ Mutation\ Frequency\ (\%) = \frac{FAM \times (1 - TP53\ R273H\ Mutation\ Frequency)}{ATTO425} \times 100\%$$

$$BRAF\ V600K\ Mutation\ Frequency\ (\%) = \frac{ROX \times (1 - TP53\ R273H\ Mutation\ Frequency)}{ATTO425} \times 100\%$$

$$RET\ M918T\ Mutation\ Frequency\ (\%) = \frac{CY5 \times (1 - TP53\ R273H\ Mutation\ Frequency)}{ATTO425} \times 100\%$$

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).

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

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
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Positive Control	The mutation frequency of the positive control is shown in the label detection value $\pm 20\%$
Negative Control	The number of mutant single-positive droplets is less than 1 (TP53 R273H single positive droplet count<1)

- Valid droplet count requirement:
Each reaction well must generate $\geq 15,000$ valid droplets. If not met, the reaction well should be retested.
- 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

When the number of single positive droplets in the 2D plot exceeds the cut-off value and meeting the quality control requirements of this kit, the sample to be tested is positive. Conversely, when the number of single positive droplets in the 2D plot is less than or equal to the cut-off value and meeting the quality control requirements of this kit, the sample to be tested is negative. The cut-off values for each site are provided in the table below:

Site	TERT C228T	TERT C250T	TP53 R273H	BRAF V600E (c.1799T>A)	BRAF K601E	BRAF V600E (c.1799_1800delinsAA)	BRAF V600K	RET M918T
Cut-off value	0	1	1 (single positive)	1	1	0	1	0

Interpretation of Test Results

Under the premises of meeting quality control requirements, each mutation should be reported separately, and the inspection results should be interpreted according to the following standards:

Test Result	Report Result	Result Interpretation
Mutant copy number = 0	Negative	No corresponding mutation detected in the sample.
Mutation frequency $\leq 0.05\%$	Negative	No corresponding mutation detected in the sample.
$0.05\% < \text{Mutation frequency} < 0.2\%*$	Indeterminate	Increase sample volume or retesting. If positive, report it as positive; if negative, report it as negative.
Mutation frequency $\geq 0.2\%$	Positive	Corresponding mutation detected.

*The sample weight is calculated as 20ng.

Product Performance Specifications

- Negative/positive coincidence rate

The test on the positive and negative reference products of the enterprise showed a 100% positive and negative coincidence rate

- Limit of detection

The test results of the enterprise limit of detection reference products are positive and the detected gene mutations are consistent with the corresponding genotypes. When the sample size on a single well is 20ng, the detailed LOD is shown in the following table:

Site	TERT C228T	TERT C250T	TP53 R273H	BRAF V600E (c.1799T>A)	BRAF K601E	BRAF V600E (c.1799_1800delinsAA)	BRAF V600K	RET M918T
LOD	0.1%	0.2%	0.1%	0.2%	0.2%	0.1%	0.1%	0.1%

3. Repeatability

The enterprise's repetitive reference samples were tested, and the same lot of reagent kits were used for three repeated tests. The test results were consistent, with all positive results and the detected gene mutations matching the corresponding genotypes.

4. Specific detection

Input 50ng of normal human genome without non-specific amplification.

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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