

Specification

16/48 tests/kit

Intended Use

This kit is intended for detecting the T790M mutation in exon 20 of the Epidermal Growth Factor Receptor (EGFR) gene in patients.

DNA target site information is shown in the table below:

Chromosome	Gene	Exon	Amino Acid Change	Nucleotide Change	COSMIC ID
Chr 7	EGFR	20	p.T790M	c.2369C>T	6240

Principle of Detection

This kit targets the allelic region of the EGFR exon 20 T790M mutation and uses specific primers to perform high-precision PCR amplification of the target sequence. Probes for mutant DNA and wild-type DNA are labeled with VIC and ROX, respectively. Using DNA from tumor tissue samples or cfDNA from peripheral blood as template, detection is performed on a digital PCR all-in-one system, enabling measurement of mutant copy number and mutation frequency.

The kit uses T790M wild-type as a reference to monitor the extraction and detection process.

Contents and Specification

No.	Content	Amount		Main Component
		16 Tests	48 Tests	
1	PCR Reaction Mix	100μL×1	300μL×1	4× DNA probe buffer, dNTP/dUTP Mix, MgCl ₂ , Cy5.5 Reference Dye, Taq DNA Polymerases
2	Primer Probe Mix	50μL×1	150μL×1	Primers and Probes
3	Positive Control	15μL×1	15μL×1	Cell line DNA mixture
4	Negative Control	15μL×1	15μL×1	Negative cell Line DNA
5	DNase/RNase-Free Water	500μL×1	500μL×1	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 (Manufacturer: Suzhou Sniper Medical Technologies Co., Ltd).

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 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 Device manufactured by Suzhou Sniper Medical Technologies Co., Ltd.

Sample Requirements

1. Applicable Sample Type:
cfDNA extracted from peripheral blood samples, or DNA extracted from 10% neutral buffered formalin-fixed paraffin-embedded tumor tissue (FFPE).
2. Sample Collection:

- a) Peripheral Blood Sample
 - Collect 10 mL venous blood into an anticoagulant tube containing EDTA (purple cap), or into a Streck Cell-Free DNA BCT tube containing K3EDTA anticoagulant and liquid cell preservative. Immediately invert gently to mix blood thoroughly with anticoagulant.
 - Blood collected in Streck Cell-Free DNA BCT tubes can be stably stored at room temperature (15–30°C) for 7 days. Blood collected in EDTA tubes can be stored at room temperature (15–30°C) for no more than 4 hours.
 - Centrifuge the collection tube at 4°C, 2000 g for 10 min. Carefully transfer the supernatant plasma into a 1.5 mL microcentrifuge (EP) tube. Then centrifuge the plasma again at 4°C, 16000 g for 10 min to remove residual blood and cellular debris. Transfer the supernatant plasma into a new 1.5 mL tube.
 - Separated plasma should be extracted immediately. If not, store as follows: At –20°C ± 5°C for no more than 3 months, allowable freeze–thaw cycles ≤ 2 times; At –80°C for no more than 1 year, allowable freeze–thaw cycles ≤ 2 times.
 - Observe the separated plasma: Light yellow indicates no hemolysis and the sample can proceed to extraction; Pink or red indicates hemolysis and subsequent procedures must not be performed.
 - After extraction, it is recommended to measure cfDNA concentration using a Qubit fluorometer, and assess purity using an Agilent 2200/4200 TapeStation system. The main fragment size distribution should be 170–200 bp, with no contamination by large-fragment genomic DNA.
 - Extracted cfDNA can be stored at –20°C ± 5°C for no more than 3 months, with allowable freeze–thaw cycles ≤ 5 times. Transport on dry ice; transport time must not exceed 110 hours.
- b) Tissue (FFPE) Sample
 - Sampling should follow pathological operating specifications. Paraffin-embedded pathological section samples should be confirmed to contain >50% tumor lesion cells (refer to the *Guidelines for Testing Technologies for Personalized Cancer Therapy*).
 - After extraction, measure DNA concentration using a Qubit fluorometer.
 - Formalin-fixed paraffin-embedded tissue samples may be transported and stored at room temperature. Extracted nucleic acids should be tested immediately; otherwise, store DNA at –20°C ± 5°C for no more than 7 months, with freeze–thaw cycles ≤ 5 times. Transport on dry ice; transport time must not exceed 110 hours.

Methods

1. Nucleic Acid Extraction

1. Select an appropriate nucleic acid extraction kit according to sample type:
 - i. For plasma extraction, it is recommended to use MagMAX™ Cell-Free DNA Isolation Kit (Life Technologies, A29319) or QIAamp Circulating Nucleic Acid Kit (Cat. No.: 55114).
 - ii. For tissue (FFPE) extraction, it is recommended to use QIAamp DNA Mini Kit (Cat. No.: 51304). Perform extraction following the corresponding IFU.
2. Use the recommended DNA quantification kit (recommended: Qubit dsDNA HS Assay Kits, Thermo Fisher Scientific, Q32851) to measure DNA concentration. DNA input requirement: > 20 ng (maximum loading volume 13 µL).

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 22µL. Prepare the reaction solution as follows:

Reaction Component	Volume
PCR Reaction Mix	6µL
Primer Probe Mix	3µL

After thoroughly mixing the above reaction solution, briefly centrifuge it and dispense it into 8-tube strips at 9µL per tube.

3. Sample addition
 - i. Control samples: Add 2 µL of control (negative control or positive control) into the 8-tube strip, and add

- ii. nuclease-free water to make a final volume of 22 μ L.
- ii. Samples to be tested: Calculate the DNA sample volume X μ L based on 20 ng DNA input, add nuclease-free water to make a final volume of 22 μ 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, 20, 24 reactions). If the number is insufficient, the remaining reactions can be replaced with 22 μ L of nuclease-free water.

3. Program Setup

1. Place the corresponding consumables according to the *Sniper All-in-one Digital PCR Device User Manual*.
2. Click "Method" to configure the amplification program as follows:

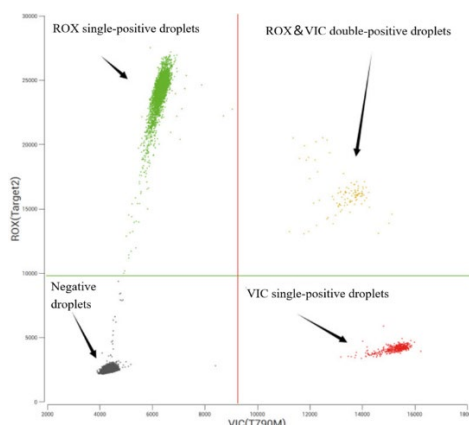
	Step	Temperature	Time	Cycle
1	Droplet spreading	60°C	300s	1
2	Enzyme activation	95°C	300s	1
3	Denaturation	95°C	15s	40
4	Annealing/Extension	63°C	30s	

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

3. Fluorescence channel selection:
 T790M wild-type: ROX
 T790M mutant: VIC
 Droplet recognition channel: Cy5.5

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" → "Well Table" 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 1/2 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:

$$\text{Copies} = \frac{\text{Valid Droplets} \times 0.6(\text{nl})}{1000} \times C$$

$$\text{Mutation Frequency} = \frac{\text{Mutant Copy Number}}{(\text{Wild-type} + \text{Mutant}) \text{ Copy Number}} \times 100\% = \frac{\text{VIC}}{\text{VIC} + \text{ROX}} \times 100\%$$

Note:

Valid Droplet: Valid droplet count.

C: Instrument detection value (copy/ μL).

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	In 2D plot: mutant single-positive droplets ≤ 1 and double-positive droplets ≤ 4 .

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 result is considered positive when the number of single-positive droplets counts > 1 in the 2D plot. If the number of single-positive droplets counts ≤ 1 and the number of double-positive droplets is ≤ 4 under valid quality control in the 2D plot, 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
Single-positive droplets ≤ 1 , and double-positive droplets ≤ 4	Negative	No EGFR T790M mutation detected in the sample.
Mutation frequency $< 0.1\%$	Positive	The result below the kit's LOD; for reference only, and retesting may yield negative with a certain probability.
Mutation frequency $\geq 0.1\%$	Positive	T790M mutation detected.

Note:

1. When the instrument readout value of (wild-type + mutant) (cp/ μL) is < 60 or the copy number (cp) is < 1000 :
 - a) if the result is negative, the sample input may be increased and retested;
 - b) if the result is positive, it may be reported as T790M mutation positive.
2. Increasing input within the tolerance range can improve sensitivity.

Product Performance Specifications

1. Concordance Rate

- a) Negative reference concordance: by testing enterprise negative reference material once per test, the negative concordance rate is 100%.

- b) Positive reference concordance: by testing enterprise positive reference material once per test, the positive concordance rate is 100%.

2. Limit of Detection (LoD)

For enterprise LoD reference material with mutation frequency 0.1% (with 20 ng input), 10 tests were performed and all results were positive.

3. Precision

For enterprise precision reference materials CV1 and CV2, 10 tests were performed, all results were positive, and the coefficient of variation of the measured mutation frequency values was $\leq 10\%$.

4. Specific Detection

- a) Interference test: The following interferents in the sample have no impact on detection: hemoglobin (2 mg/mL), bilirubin (350 $\mu\text{M/L}$), triglycerides (37 mmol/L), cisplatin (12 mg/L), EDTA (2 mg/mL), cholesterol (6.47 mmol/L), ethanol (21.7 mmol/L). The detection results are not affected.
- b) Cross-reactivity: No cross-reaction with non-human genomes including *E. coli* and yeast, pulmonary infection-related microbial genes (genomic DNA of *Mycobacterium tuberculosis complex*, *Haemophilus influenzae*, *Streptococcus pneumoniae*), or other EGFR mutation types. The assay can tolerate 50 ng 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

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Instruction Approval and Revision Date

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