

Specification

48 tests/kit

Product Description

This kit is intended for in vitro detection of relative copy number variation of the HER2 gene in formalin-fixed, paraffin-embedded (FFPE) human breast tissue sections. It is intended for use by qualified personnel in molecular and cancer research to investigate HER2 gene status. For Research Use Only. Not for use in diagnostic procedures.

The human epidermal growth factor receptor 2 (HER2) gene, located on chromosome 17q21, encodes a transmembrane receptor-like protein with a molecular weight of approximately 185 kDa and intrinsic tyrosine kinase activity. The HER2 protein interacts with other members of the HER family and their ligands, participating in intercellular signal transduction pathways that regulate cell growth, differentiation, and proliferation.

Studies have reported that HER2 gene amplification is observed in approximately 15–20% of breast tissue samples. Such amplification is often associated with distinct biological characteristics. In research settings, HER2 protein expression is commonly assessed using immunohistochemistry (IHC), while HER2 gene copy number variations are evaluated through in situ hybridization techniques such as FISH.

HER2 signaling pathways and their targeted inhibition remain critical topics in molecular oncology research. Numerous HER2-targeting compounds, including antibodies, antibody-drug conjugates, and small-molecule inhibitors, have been developed for investigational purposes. These tools are widely used to study HER2-driven mechanisms in breast cancer models.

Data generated using this kit are for research reference only. Users should carefully consider sample type, experimental conditions, and other variables when designing studies and interpreting results.

This product is not intended for use in clinical diagnosis, disease evaluation, therapeutic decision-making, or any medical purpose involving human subjects.

Principle of Detection

This kit is designed to detect the copy number variation of the HER2 target gene and three human reference genes (Gene E, Gene R, and Gene C). It utilizes specific primers and TaqMan fluorescent probes targeting these genes. Using DNA extracted from FFPE tissue samples, the assay is performed on the All-in-one Digital PCR System (DQ24 Plus), enabling quantitative detection of the HER2 gene (detected in the HEX fluorescence channel) and the reference genes—Gene E (FAM channel), Gene R (ROX channel), and Gene C (Cy5 channel). These reference genes are located on chromosomes 2, 10, and 17, respectively, helping to reduce the risk of false-negative or false-positive results caused by chromosomal abnormalities.

By calculating the ratio of HER2 gene copy number to the reference gene copy number and comparing it with a predetermined threshold, users can evaluate the HER2 amplification status in the tested sample.

The reaction system is partitioned into approximately 20,000 microdroplets, with each droplet functioning as an independent PCR microreactor. During the PCR process, the Taq DNA polymerase cleaves the hybridized fluorescent probe via its 5' to 3' exonuclease activity while extending the DNA strand. This cleavage separates the fluorescent reporter dye from the quencher, generating a fluorescent signal upon excitation with a specific light source. By detecting the fluorescence signal of each droplet, the target gene copy number in the entire reaction can be calculated based on Poisson distribution.

Contents and Specification

No.	Content	Amount	Main Component
1	dPCR Master Mix	72μL×3	10×PCR Buffer, dNTPs, Taq DNA Polymerase, MgCl ₂ , Restriction Enzyme
2	Primer Probe Mix	40μL×3	Primers and Probes
3	Positive Control	15μL×3	HER2-Positive Cell Line gDNA
4	Negative Control	15μL×3	Negative Control gDNA
5	DNase/RNase-Free Water	500μL×3	DNase/RNase-Free Water

Note:

- The components in different lots of kits cannot be mixed.
- The kit does not contain 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 (Manufacturer: Suzhou Sniper Medical Technology 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 Instruments manufactured by Suzhou Sniper Medical Technology Co., Ltd.

Sample Requirements

- Formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples fixed in 10% neutral buffered formalin, with a tumor content of no less than 10%.
- FFPE Tissue Requirements:
 - Sample collection:
Tumor tissue specimens intended for HER2 gene amplification analysis should be processed and preserved according to standard laboratory procedures after fixation in 10% neutral buffered formalin and paraffin embedding.
For each sample, ten sections of 5–10 μm thickness should be prepared (tissue size: 1×1 cm²). One section should be used to determine the tumor cell percentage, using commonly accepted histological methods. The remaining sections (at least three) should be used for DNA extraction.
To avoid cross-contamination, special care must be taken during sectioning and tumor tissue scraping to ensure samples from different cases are processed separately.
 - DNA quality control: After nucleic acid extraction, DNA concentration and purity should be measured using a micro-volume spectrophotometer.
 - Transportation & storage:
FFPE tissue samples may be stored and transported at room temperature, with a storage period not exceeding 4 years.

Extracted DNA should be tested immediately. If not used immediately, store at $-20 \pm 5^{\circ}\text{C}$ for no longer than 7 months. A maximum of 5 freeze-thaw cycles is allowed. DNA samples should be shipped on dry ice, and total shipping duration ≤ 110 hours.

Methods

1. Nucleic Acid Extraction

1. This kit does not include nucleic acid extraction components. DNA should be extracted from FFPE tissue sections using a nucleic acid extraction kit manufactured by Suzhou Sniper Medical Technology Co., Ltd. Please refer to the manufacturer's instructions for detailed extraction procedures.
2. It is recommended to use a micro-volume spectrophotometer to assess the concentration and purity of the extracted DNA. The DNA concentration $> 1 \text{ ng}/\mu\text{L}$, and the OD260/OD280 ratio > 1.6 . The amount of DNA input per reaction should be calculated based on the measured concentration and should meet the requirement of 10 ng per reaction.

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 μL . Prepare the reaction solution as follows:

Reaction Component	Volume
dPCR Master Mix	4.5 μL
Primer Probe Mix	2.5 μL
Sample	1-12.56 μL (10ng)
DNase/RNase-Free Water	Up to 22 μL
Total	22 μL

3. Sample addition
 - i. **Control samples:** Take 5 μL 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 μL .
 - ii. 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 μL 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 $^{\circ}\text{C}$	300s	1

2	Enzyme activation	95°C	300s	1
3	Denaturation	95°C	20s	50
4	Annealing & Extension	58°C	30s	
5	Final Extension & Imaging	55°C	1200	1

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

3. Fluorescence channel selection:

Fluorescence Channel	HEX	FAM	ROX	CY5
Target	HER2	Reference Gene E	Reference Gene R	Reference Gene C
Droplet Recognition Channel: CY5.5				

4. Result Analysis

- Result review and threshold line determination:
 - After dPCR reaction completion, navigate to "Analysis Plot" → "1D dot Plot" or "2D dot Plot" → "Plate Map" to view amplification results for each well.
 - 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".
 - Verify all reaction wells to ensure the threshold line does not intersect any droplet clusters.
 - 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").
 - Positive droplet count and concentration results will be generated based on the threshold line.
- 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.
- Selection of Detection Samples:
Samples with reference gene copy numbers > 3,500 should be selected for analysis. If the reference gene copy number is ≤ 3,500, the sensitivity of the assay may be affected. In such cases, increasing the input DNA amount is recommended to improve detection performance.
- Result calculation:
Copy Number calculation:

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

Note:

Nb Droplet: Valid droplet count.

C: Instrument detection value (copy/μL).

Reference Gene CN copy number calculation:

Reference Gene CN Copy Number

= Median(Reference Gene E Copy Number, Reference Gene R Copy Number, Reference Gene C Copy Number)

The kit reports results as mutation frequency, calculated as:

$$\text{Copy Number Ratio} = \frac{\text{HER2 Copy Number}}{\text{Reference Gene CN Copy Number}}$$

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	The Copy Number Ratio of the positive control is 1.4 - 1.7.
Negative Control	The Copy Number Ratio of the negative control < 1.07

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

A total of 145 FFPE clinical samples were collected to establish the positive cutoff value. Based on the ROC curve analysis of the detection results, the cutoff ratio of HER2 gene copy number to reference gene copy number was determined to be 1.6. A sample is considered HER2 amplification positive when the ratio ≥ 1.6 , and HER2 amplification negative when the ratio < 1.6.

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
Copy Number Ratio < 1.6	Negative	HER2 gene amplification is negative.
Copy Number Ratio ≥ 1.6	Positive	HER2 gene amplification is positive.

Limitation of the Detection Method

1. Data generated using this kit are for research reference only. Users should carefully consider sample type, experimental conditions, and other variables when designing studies and interpreting results.
2. Test results are affected by the quality of sample collection, processing, transportation, and storage. Any error in these steps may lead to inaccurate results.
3. A negative result does not completely rule out the possibility of HER2 gene amplification. Low tumor content or excessive nucleic acid degradation in the sample may lead to false-negative results.
4. Tumor heterogeneity may exist within the sample. Sampling from different regions of the same tissue may yield different results.
5. This kit is intended only for the analysis of FFPE tumor tissue samples fixed in 10% neutral buffered formalin. Other sample types are not validated for use with this product.

Product Performance Specifications

1. Concordance Rate

Negative Reference Samples: The kit was tested with five enterprise-provided negative reference samples at both low and high concentrations. All results were negative for HER2 gene amplification, with a negative concordance rate of 100%.

Positive Reference Samples: The kit was tested with three enterprise-provided positive reference samples (weak, moderate, and strong positives). All results were positive for HER2 gene amplification, with a positive concordance rate of 100%.

2. Limit of Detection (LoD)

A total of 152 negative samples were tested, and the upper limit of the reference range for negative samples was determined to be 1.069 based on the mean value analysis.

Serial dilutions were prepared with HER2/reference gene ratios ranging from 1.0 to 6.0, and tested in replicates using the kit (10 ng input DNA). Using 1.07 as the decision threshold, the LoD was defined as the lowest concentration that achieved $\geq 95\%$ positive detection rate. The result showed that a concentration of 1.15 met this requirement. Therefore, the LoD of the kit is 1.15 (HER2/reference gene copy number ratio).

3. Precision

Three reference samples were tested in 10 replicates:

- Reference materials J1 and J2 were consistently HER2 amplification positive, with coefficient of variation (CV) values for the HER2/reference gene copy number ratio $\leq 10\%$.
- Reference material J3 was consistently HER2 amplification negative.

4. Specific Detection

- Interference Testing: The assay was not affected by endogenous substances such as paraffin ($\leq 1\%$ V/V) and formalin ($\leq 0.005\%$ V/V). Additionally, common therapeutic agents including amoxicillin, doxorubicin, paclitaxel, cyclophosphamide, trastuzumab, pertuzumab, and lapatinib—at concentrations up to $2.5\times$ clinical levels—had no observable interference.
- Cross-Reactivity Testing: No cross-reactivity was observed with *Escherichia coli* or *Staphylococcus aureus*.

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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