4448 Ammendale Rd Beltsville, MD 20705, USA
Tel# +1 (408) 580-1396
E-mail: info@genebiosolution.com
Website: www.genebiosolution.com

Product Cat. No.: GBS-008

For Research Use Only.

TOP2A gene amplification detection kit Instructions Manual

[Product Name] TOP2A gene amplification probe detection kit.

[Package Specifications] 10 Tests/box.

[Intended Usage]

This kit uses fluorescence in situ hybridization to detect TOP2A gene status in breast cancer tissues, and the test samples are paraffin embedded specimens of surgical or biopsy tissues of breast cancer. This kit is only applicable to the detection of TOP2A gene status and provides doctors with diagnostic auxiliary information.

Breast cancer is the most common malignant tumor in human beings, and also one of the major malignant tumors in women. In recent years, the incidence rate of breast cancer in China is on the rise. At present, invasive breast cancer has been regarded as a systemic disease, and its prognosis judgment and prognostic related biomarkers have attracted much attention. Research on breast cancer shows that when TOP2A gene is positive in breast cancer tissue, lymph node metastasis is more serious; Patients with abnormal TOP2A gene predicted a shorter relapse-free survival period, and patients with TOP2A gene deletion had a worse prognosis. Cytotoxic chemotherapy drugs represented by anthracyclines have been widely used in neoadjuvant chemotherapy and postoperative chemotherapy for breast cancer. They benefit some patients and bring great pain to patients due to adverse cardiac reactions. However, there is no clear biological indicators to predict their efficacy. In the study of advanced breast cancer, TOP2A gene abnormality was found to be significantly related to protein expression and tumor cell sensitivity to anthracyclines' drugs. Compared with CMF regimen, CEF regimen (containing anthracyclines) can reduce the recurrence risk by 61% and the death risk by 51% for patients with TOP2A abnormalities, while CEF regimen can only reduce the recurrence risk by 6% and the death risk by 10% for patients without TOP2A abnormalities.

Therefore, the detection of TOP2A gene status has guiding significance for the treatment and prognosis of breast cancer.

This kit has not been clinically verified in combination with TOP2A gene targeted therapeutic drugs, but only for the detection performance of TOP2A gene.

[Detection Principle]

This kit is based on fluorescence in situ hybridization technology. One kind of nucleotide of nucleic acid probe is labeled with fluorescein. The detected target gene and the used nucleic acid probe are homologous and complementary. After denaturation, annealing and renaturation, the two can form a hybrid of target gene and nucleic acid probe. Through fluorescence detection system, qualitative, quantitative or relative positioning analysis of the gene to be tested is carried out under the microscope.

This kit uses the orange probe (TOP2A probe) labeled with rhodamine fluorescein (RHO) to detect TOP2A gene, and the green probe (CEP17 probe) labeled with fluorescein isothiocyanate (FITC) to detect the centromere sequence of chromosome 17. The two probes can be combined with the target detection site by in situ hybridization. The signal number corresponding to CEP17 probe reflects the number of chromosome 17 at the target site, while the signal number of TOP2A probe corresponds to the status of TOP2A gene at the target site. The TOP2A gene status of the tissue to be tested can be determined by the ratio of orange signal to green signal fluorescence number.

[Product Main Components]

The kit consists of TOP2A orange probe and CEP17 green probe, as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
TOP2A/CEP17 dual color probe	100μL/Tube	1	TOP2A orange probe ; CEP17 green probe

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[Storage conditions & Validity]

Keep sealed away from light at -20°C±5°C. The product is valid for 20 months. Avoid unnecessary repeated freezing and thawing that should not exceed 10 times. After opening, within 24 hours for short-term preservation, keep sealed at 2~8°C in dark. For long-term preservation after opening, keep the lid sealed at -20°C±5°C away from light. See the label of the kit for the production date and expiration date.

[Applicable Instruments]

Fluorescence microscopy imaging systems, including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

[Sample Requirements]

1. Applicable specimen type: paraffin embedded specimen of surgical resection or biopsy tissue of breast cancer.
2. The specimen should be fixed with 4% neutral formalin fixation solution within 1 hour after being isolated. The fixation time should be 6~72 hours. After the specimen is fixed, it is often dehydrated and paraffin embedded.
3. The thickness of paraffin section will affect the experimental results, and the section thickness is 4-5μm is appropriate.
4. For paraffin embedded tissue samples of breast cancer, representative tumor tissue wax blocks should be selected and confirmed by HE staining.

It is recommended to select paraffin-embedded tissue samples with shorter preservation time (within 5 years).

[Testing Method]

1 . Related reagents

The following reagents are required for the experiment but not provided in this kit

① 20×SSC (sodium citrate buffer), pH 5.3±0.2

Sodium chloride	176g
Sodium citrate	88g

Weigh 176g of sodium chloride and 88g of sodium citrate, dissolve in 800mL of deionized water, adjust the pH to 5.3±0.2 at room temperature, and complete to 1 L with deionized water. High-pressure steam sterilization, stored at 2~8°C, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

② 2×SSC, pH 7.0±0.2

Take 100mL of the above 20xSSC, dilute with 800mL deionized water, mix, adjust the pH to 7.0±0.2 at room temperature, complete to 1L with deionized water, stored at 2~8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

③ Ethanol Solution: 70% ethanol, 85% ethanol

Dilute 700ml, 850ml of ethanol with deionized water to 1L. The shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

④ Protease K

Protease K stock solution (20mg/mL): Weigh 0.1g of proteinase K dry powder, dissolve in 5mL 2xSSC (pH value 7.0). Gently mix the solution till completely dissolved, and store at -20°C. Shelf life is 6 months.

Protease K working solution (200μg/mL): Dissolve 0.8mL Proteinase K stock solution in 80mL 2×SSC (pH value 7.0), mix well and the solution is ready for immediate use.

⑤ 0.3% NP-40/0.4xSSC solution, pH 7.0 ~ 7.5

NP-40	0.6mL
20xSSC	4mL

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Take 0.6mL NP-40 and 4mL 20×SSC, add 150mL deionized water, mix, adjust the pH to 7.0 ~ 7.5 at room temperature, with deionized water complete to a volume of 200mL. Stored at 2~8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑥0.1% NP-40/2×SSC solution, pH 7.0 ± 0.2

NP-40	0.2mL
20xSSC	20mL

Take 0.2mL NP-40 and 20mL 20×SSC, add 150mL deionized water, mix, adjust the pH 7.0±0.2 at room temperature, with deionized water complete to a volume of 200mL. Stored at 2~8oC, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑦ Diamidinyl phenylindole (DAPI) counterstain

Use commercially available anti-quenching DAPI counterstain.

⑧ Xylene.

2. Pretreatment

It is recommended to select specimens with known positive and negative TOP2A gene amplification as controls.

①Sectioning: Place on clean slides tissue section fixed in neutral formalin and paraffin-embedded.

②Heating slices: Place the slices in the heating machine and heat overnight at 65°C.

③Dewaxing: Immerse the tissue sections in xylene for 10 minutes, dewax, repeat once, and then immediately immerse in 100% ethanol for 5 minutes.

④ Rehydration: At room temperature, place in 100% ethanol, 85% ethanol, and 70% ethanol for 2 minutes each the tissue sections, immediately immerse in deionized water for 3 minutes. Take out the slices and use a lint-free tissue to absorb excess water around the tissue.

⑤ Water treatment: Soak tissue sections in deionized water at 95°C for 30 to 40 minutes (deionized water is applied in a water bath to preheat).

⑥ Washing: At room temperature, soak the tissue sections in 2xSSC solution and rinse twice for 5 minutes each.

⑦ Proteinase K treatment: Soak the tissue sections in proteinase K working solution and treat at 37°C for 5-30 minutes.

⑧ Washing: At room temperature, soak the tissue sections in 2xSSC solution and rinse twice for 5 minutes each.

⑨ Dehydration: Placed the tissue sections in 70% ethanol, 85% ethanol and 100% ethanol for 2 minutes each, and then dry naturally.

3 . Denaturation and Hybridization

The following operations should be performed in a darkroom.

① Take the dual-color probe. Mix and centrifuge briefly. Take 10μl droplet in the cell and drop in the hybridization zone, immediately cover 2mmx22mm glass slide area; spread evenly without bubbles the probe under the glass slide covered area and seal edges with rubber (edge sealing must be thorough to prevent dry film from affecting the test results during hybridization).

② Place the glass slides in the hybridization instrument, denature at 83°C for 5 minutes (the hybridizer should be preheated to 83°C) and hybridize at 42°C for 2 to 16 hours.

4 . Washing

The following operations should be performed in a darkroom.

① Take out the hybridized glass slides, remove the rubber on the coverslip and immediately place the slides in a 0.3% NP-40/0.4x SSC solution at 67°C. Shake for 1-3 seconds, remove the coverslip and continue to soak the glass slides for 1-2 minutes.

② At room temperature, place the slices in 0.1%NP-40/2xSSC solution, oscillate for 1-3 seconds and soak for 1-2 minutes.

③ At room temperature, place the slices in 70% ethanol, soak for 1-3 minutes, and naturally dry in the dark.

5. Counterstaining

The following operations should be performed in a darkroom.

Dip 10~15μL of DAPI counterstain into the hybridization area of the glass slide, immediately cover and place in dark for 10~20 minutes, and then use the suitable filter to observe the sections under the fluorescence microscope.

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6. FISH results observation

Place the stained sections under a fluorescence microscope and the area of the breast cancer cells is first confirmed under a low magnification objective (10x); under magnification objective (40x) a uniform cells distribution is observed; then the nucleus size uniformity, nuclear boundary integrity, DAPI staining uniformity, no nuclei overlapping, cells clear signal are observed in the high magnification objective (60x, 100x). Select randomly 20 tumor cells at least and count the orange and green signals in the nuclei.

[Positive judgment value or reference interval]

1. Signal classification and counting

The number of TOP2A signals and the number of signals of CEP17 in 20 tumor cells were observed and recorded (Table 3). The total number of TOP2A signals indicates the total TOP2A copy number, and the total number of CEP17 signals indicates the total number of chromosomes 17 in the target area. Calculate the ratio value (Ratio = Total number of TOP2A signals / Total number of CEP17 signals).

2. Breast cancer FISH result determination

① When Ratio ≥ 2.0, the tissue can be considered as TOP2A gene amplification positive.

② When the ratio is less than 0.8, the tissue can be considered as TOP2A gene deletion positive.

③ When 0.8 ≤ Ratio<2.0, it can be considered that the tissue is abnormally negative for TOP2A gene.

④ When orange signal is connected in clusters or Ratio>20, it cannot be counted, and it is directly regarded as TOP2A gene amplification.

When the ratio value is between 0.7~0.9 or 1.8~2.2, it is necessary to carefully interpret the results, increase the number of cells, and recalculate the ratio value. If the hybridization signal is weak or the background is strong, it is considered as hybridization failure, and the experiment needs to be repeated.

[Product performance index]

1.Fluorescence signal intensity: after the probe is effectively hybridized with the karyotype sample, it should emit a fluorescence signal that can be recognized by the naked eye under the fluorescence microscope.

2.Sensitivity: detect karyotype samples, analyze the chromosomes of 50 cells in metaphase, and at least 98 chromosomes 17 show 1 orange fluorescence signal and 1 green fluorescence signal.

3.Specificity: Analyze the chromosomes of 50 cells in metaphase, at least 98 chromosomes 17 show a specific orange fluorescence signal in the target region and a specific green fluorescence signal in the centromere region.

Precautions]

1.Please read this manual carefully before testing. The testing personnel should receive professional technical training. The signal counting personnel must be able to observe and distinguish orange and green signals.

2. When testing clinical samples, when it is difficult to count the hybridization signals and the samples are not enough to repeat the test, the test will not provide any test results. If the number of cells is not enough for analysis, the test will not provide test results.

3.The formamide and DAPI dye used in this experiment have potential toxicity or carcinogenicity, so it is necessary to operate in a fume hood, wear masks and gloves to avoid direct contact.

4.The results of this kit will be affected by various factors of the sample itself, as well as by the restriction of enzyme digestion time, hybridization temperature and time, operating environment and limitations of current molecular biological technology, which may lead to incorrect interpretation results. Users must understand the potential errors and limitations of accuracy that may exist in the detection process.

5. All chemicals are potentially dangerous. Avoid direct contact. The used reagent box is clinical waste and should be properly disposed.

6.This product is only used for in vitro diagnosis.