Product Catalogue Number MBI-FP-A008

TOP2A Gene Amplification Probe Detection Kit

[Product Name] TOP2A Gene Amplification Probe Detection Kit (Fluorescence In Situ Hybridization Method).

[Intended Use]

This kit uses fluorescence in situ hybridization (FISH) to detect the TOP2A gene status in breast cancer tissues in vitro, and the test sample is a paraffin-embedded specimen of breast cancer surgery or biopsy tissue. This kit is only suitable for the detection of the TOP2A gene status and provides physicians with auxiliary information for diagnosis.

Breast cancer is one of the most common malignant tumors in human beings and one of the main malignant tumors in women. In recent years, the incidence of breast cancer is on the rise. At present, invasive breast cancer has been regarded as a systemic disease, and its prognostic judgement and prognostic biomarkers have attracted much attention. Studies on breast cancer have shown that lymph node metastasis is more serious when TOP2A gene is expressed positively in breast cancer tissues; patients with abnormal TOP2A gene indicate shorter recurrence-free survival and worse prognosis in patients with TOP2A gene deletion. Cytotoxic chemotherapeutics represented by anthracyclines are widely used in neoadjuvant chemotherapy and post-operative chemotherapy for breast cancer. They benefit some patients and cause great pain due to adverse cardiac reactions. However, there is no clear biological indicators to predict their efficacy. In the study of advanced breast cancer, it was found that TOP2A gene abnormality was significantly associated with protein expression and sensitivity of cancer cells to anthracyclines. Compared with CMF regimen, CEF regimen (including anthracyclines) can reduce recurrence risk by 61% and mortality risk by 51% in patients with TOP2A abnormalities, while CEF regimen can only reduce recurrence risk by 6% and mortality risk by 10% in patients without TOP2A abnormalities. Therefore, the detection of TOP2A gene status has guiding significance for the treatment and prognosis of breast cancer.

The kit has not been clinically validated in combination with the TOP2A gene-targeted therapeutic drug. Only the TOP2A gene detection performance was verified.

[Detection Principle]

This kit is based on fluorescence in situ hybridization, a nucleotide of the nucleic acid probe is labeled with fluorescein, and the detected target gene is homologously complementary to the nucleic acid probe used. After denaturation, annealing and renaturation, a hybrid of the target gene and the nucleic acid probe can be formed, and the gene to be detected is subjected to qualitative, quantitative or relative localization analysis under the microscope by a fluorescence detection system.

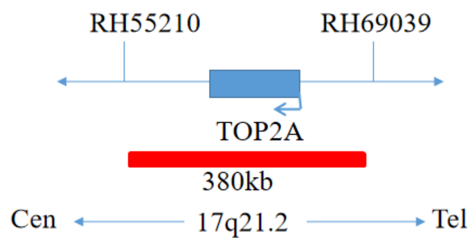
This kit uses the rhodamine fluorescein (RHO)-labeled orange-red probe (TOP2A probe) to detect the TOP2A gene, and the fluorescein isothiocyanate (FITC)-labeled green probe (CEP17 probe) for detection of chromosome 17. The two probes can be combined with the target detection site by in situ hybridization. The number of signals corresponding to the CEP17 probe reflects the number of chromosome 17 at the target site, and the number of signals of the TOP2A probe corresponds to the state of the target site TOP2A gene. By comparing the ratio of the orange-red signal to the green signal fluorescence number, the state of the TOP2A gene to be detected can be determined.

[Product Main Components]

The kit consists of a TOP2A orange-red probe and a CEP17 green probe hybridization solution 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-red probe CEP17 green probe



[Storage conditions & Validity]

Keep sealed away from light at -20°C± 5°C. The product is valid for 12 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.

[Applicable Instruments]

Fluorescence microscopy imaging system, including fluorescence microscopy and filter groups suitable for DAPI, Green and Orange.

[Sample Requirements]

Tissue sample

1. Applicable specimen types: Breast cancer resection or paraffin-embedded specimens of biopsy tissue.
2. The specimens should be fixed with 4% neutral formalin within 1 hour after isolation. The suitable time for fixation is 6-72 hours. After fixation, the specimens should be dehydrated and embedded in paraffin.
3. The paraffin sections thickness affect testing results. The thickness of the slice should be 4-5µm.
4. The paraffin-embedded tissue specimens of breast cancer should be selected representative paraffin-embedded tissues and confirmed by HE staining.
5. It is recommended to select paraffin-embedded tissue specimens with shorter preservation time (within 5 years).

[Testing Method]

1. Related Reagents

① 20×SSC, pH 5.3±0.2

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 Pepsin

Pepsin stock solution: Weigh 5g of pepsin dry powder dissolved in 100mL of distilled water, gently shake until complete dissolution, and stored at -20°C, the shelf life is 6 months.

Pepsin working solution (0.5%): Take 5 mL of pepsin stock solution dissolved in 45 mL HCL solution (pH=2), and mix thoroughly.

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

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.

⑥ Diamidinyl phenylindole (DAPI) counterstain

Use commercially available anti-quenching DAPI counterstain.

2. Pre-hybridization treatment

① ***Sectioning***: Neutral formalin fixed paraffin-embedded tissue sections are placed on a clean glass slides.
② Heating or baking: The tissue slides are placed on the baking machine 65°C overnight (30min at 80°C baking for old slices).
③ ***Dewaxing***: Tissue sections are soaked during 10 minutes in xylene dye tank for dewaxing, repeated once, and then immediately immersed in 100% ethanol for 5 minutes.
④ ***Rehydration***: At room temperature, the tissue slices are placed in 100% ethanol, 85% ethanol and 70% ethanol for 2 minutes, and then immersed in deionized water for 3 minutes. After taking out the slices, remove by absorption the excess moisture around the tissue slices with sterile clean tissue paper.
⑤ ***Washing***: Under 95°C water bath, the tissue slices are soaked in deionized water for 30 to 40 minutes (deionized water is preheated by water bath).
⑥ ***Washing***: At room temperature, the tissue sections are soaked in 2xSSC solution, rinse twice for 5 minutes each.
⑦ ***Enzyme digestion***: the tissue slides are immersed in the protease working solution, at 37°C for 5 to 30 minutes (the protease working solution is now ready for use, and should be discarded after one use).

Protease action time depends on the slice thickness. In order to achieve proteins full digestion and do not affect the tissue morphology, proteases can also digest proteins surrounding the target DNA, increasing the probe and the target DNA binding opportunities and improving the hybridization rate. If the protease concentration is too high, the digestion time subsequently will be too long; or if the incubation temperature is too high, it will destroy the cell structure, resulting in tissue sections falls off, disappearance of nuclei or unclear nuclei. Insufficient protease digestion will affect tissue permeability, signal intensity and hybridization rate with too strong auto-fluorescence under the microscope.

⑧ ***Washing***: At room temperature, the tissue sections are soaked in 2xSSC solution, rinse twice for 5 minutes each.
⑨ ***Dehydration***: The tissue slices are placed in order in 70% ethanol, 85% ethanol and 100% ethanol for 2 minutes each, take out and air dry.

3. Denaturation and Hybridization

The following operations should be performed in a darkroom.
① Take the TOP2A probe at static room temperature for 5 minutes. Briefly centrifuge (1-2s) after manually mixing the probe (do not use vortex/swirl or shaker instrument/oscillator). Take 10µl droplet in the cell and drop in the hybridization zone, immediately cover 22mmx22mm 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 slide in the hybridization instrument, denature at 85°C for 5 minutes (the hybridizer should be preheated to 85°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 immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.
② Place the slides in a 2×SSC at room temperature for 1 min.
③ Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution and wash for 2min.
④ Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

5. Dyeing

The following operations should be performed in a darkroom
10µl DAPI compound dye is dropped in the hybridization area of the glass slide and immediately covered. The suitable filter is selected for glass slide observation under the fluorescence microscope.

6. FISH results observation

Place the stained sections under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10×); under magnification objective (40×) 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 (100x). At least 50 tumor cells are randomly selected and the red and green signals in the nucleus are counted.

[Positive Value or Reference Interval]

1. Signal classification and counting

Observe and randomly count 60 cells (the cells with no signal or only one color signal in the nucleus are not counted), and count the number of orange-red signal (TOP2A) and green signal (CEP17) respectively. The sum of the orange-red signals indicates the total TOP2A number of signals. The sum of the green signals, represents the total number of CEP17 signals, and the Ratio value is calculated as: Ratio = Total number of TOP2A signals in the nucleus / Total number of CEP17 signals in the nucleus.

2. FISH results evaluation

a). Ratio ≥ 2.0, the tissue is considered to be positive for TOP2A gene amplification.
b). Ratio <0.8, the tissue is considered to be positive for the TOP2A gene deletion.
c). 3 0.8 ≤ Ratio < 2.0, the tissue is considered to be abnormally negative for the TOP2A gene.
d). When the orange-red signal is connected to a cluster or Ratio >2.0, it can be counted and directly regarded as TOP2A gene amplification.
When the Ratio value is between 0.7-0.9 or 1.8-2.2, the result should be carefully interpreted. Counting cells can be increased and Ratio value can be recalculated. If the hybridization signal is weak or the background is strong, it will be considered as hybridization failure and the experiment needs to be re-conducted.

[Testing Method Limitations]

This kit is used for paraffin-embedded specimens of breast cancer resection or biopsy tissues. It is not recommended for other tissues. The detection ability of paraffin tissue samples with too long storage time cannot be evaluated according to this instruction; it should be operated according to the procedures provided in this manual. Procedure change may alter the results of the test. This kit only detects the status of the TOP2A gene and cannot be used as the sole basis for treatment, prognosis or other clinical management of breast cancer patients. Comprehensive evaluation should be made on the basis of patient medical history and other diagnostic results.

[Product Performance Index]

1. Fluorescence signal intensity: After hybridization with karyotype samples, the probe should emit fluorescence signals that can be recognized by naked eyes under fluorescence microscopy.
2. Sensitivity: Karyotype samples were detected and chromosomes of 50 cells in metaphase were analyzed. At least 98 chromosomes 17 showed an orange fluorescence signal and a green fluorescence signal.
3. Specificity: Chromosomes of 50 cells in metaphase were analyzed. At least 98 chromosomes 17 showed 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 undergo professional technical training. The signal counting personnel must be able to observe and distinguish the orange-red and green signals.

2. When it is difficult to count the hybridization signals and the sample not enough to repeat the test, the test will not provide any results. If the cell size is insufficient for analysis, similarly, the test will not provide test results.

3. The formamide and DAPI counterstain used in this experiment are potentially toxic or carcinogenic. They need to be operated in a fume hood, wearing masks and gloves to avoid direct contact.

4. The results of this kit will be affected by various factors of the sample itself, but also by the restrictions of enzyme digestion time, hybridization temperature and time, operating environment and the limitations of current molecular biology technology, which may lead to wrong interpretation results. Users must be aware of potential errors and limitations of accuracy in the detection process.

5. All chemicals are potentially hazardous and avoid direct contact. The used kits are clinical waste and should be disposed of properly.

[Reference]

[1]. K. E. Olsen et al. Amplification of HER2 and TOP2A and deletion ofTOP2A genes in breast cancer investigated by newFISH probes, ActaOncologica 43 (2004)

[2]. K. V. Nielsen et al. The value of TOP2A gene copy number variation asa biomarker in breast cancer: Update of DBCG trial89D, ActaOncologica, 2008; 47: 725734

[3]. Zaczek, Markiewicz, Seroczynska et al. Prognostic Significance of TOP2A Gene Dosage in HER-2-NegativeBreast Cancer, The Oncologist 2012;17:1246–1255

[4]. Järvinen TA, Tanner M, Bärlund M, et al. Characterization of topoisomerase II alpha gene amplification and deletion in breast cancer. Genes Chromosomes Cancer. 1999 Oct; 26(2):142-50.

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