Cytowish Ltd
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Your authentic partner in molecular cytogenetics

NTRK1/NTRK2/NTRK3 Gene Break Apart Probe Detection Kit

(CW-231)

Intended use

The reagent carries out in situ hybridization staining on the basis of routine staining to provide doctors with auxiliary information for diagnosis. The test results are only for clinical reference and should not be used as the only basis for clinical diagnosis. Clinicians should comprehensively judge the test results in combination with the patient's condition, drug indications, treatment response and other laboratory test indicators.

Product composition

The kit consists of one of NTRK1, NTRK2 or NTRK3 dual color probe.

Component name	Catalogue No.	Specifications	Quantity	Main components
NTRK1dual color probe	CW231-1	100 μL/Tube	1	NTRK1 Orange probe, NTRK1 Green probe
NTRK2dual color probe	CW-231-2	100 μL/Tube	1	NTRK2 Orange probe, NTRK2 Green probe
NTRK3dual color probe	CW-231-3	100 μL/Tube	1	NTRK2 Orange probe, NTRK2 Green probe

Storage condition

This kit is shipped below 0°C. 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 sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

Sample requirements

1. Applicable specimen types: Paraffin-embedded specimens for surgical resection or biopsy.

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2. Tissue should be fixed with 4% neutral formaldehyde fixation solution within 1 hour after in vitro, and the tissue should be fixed by conventional dehydration and paraffin embedding.

Sample pretreatment

It is recommended to use CytoWish's FISH pretreatment reagent (catalogue number CW-CT-FISH).

Denaturation and hybridization

The following operations need to be carried out in the darkroom.

1. Take the probe at room temperature for 5 minutes. Briefly centrifuge manually (do not use vortex or shaker instrument). 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.

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 into 2xSSC for 5 seconds, and gently remove the coverslip.
2. Place the glass slides in 2xSSC at room temperature.
3. Remove and immerse the slides in a 0.3% NP-40/0.4×SSC solution preheated at 68°C for 2 min.
4. Immerse the glass slides in deionized water at 37°C for 1min, and dry naturally in the dark.

Counterstaining

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.

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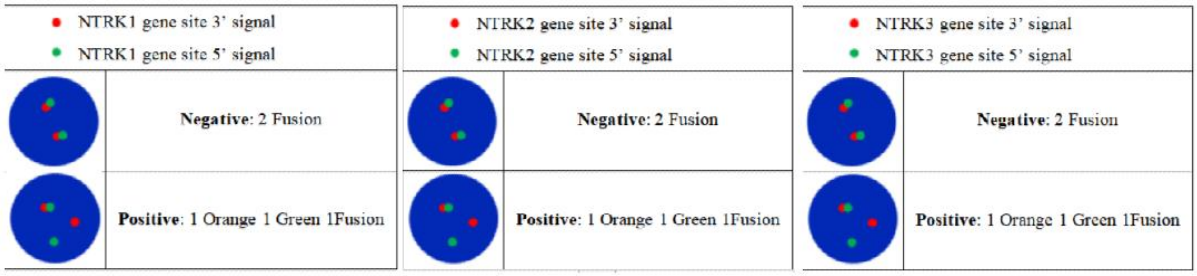
FISH results observation

Place the stained slide under a fluorescence microscope and the cells area 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 (100x).



Precautions

1. Please read this manual carefully before testing. The testing personnel shall receive professional technical training. The signal counting personnel must be able to observe and distinguish orange red and green signals.
2. When testing clinical samples, if it is difficult to count the hybridization signals and the samples are not enough to repeat the retest, the test will not provide any test results. If the amount of cells is insufficient for analysis, again, the test will not provide test results.
3. The formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity, so they need to be operated in the fume hood and wear 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 limited by enzyme digestion time, hybridization temperature and time, operating environment and limitations of current molecular biology technology, which may lead to wrong results. The user must understand the potential errors and accuracy limitations that may exist in the detection process.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical wastes and should be properly disposed of.
6. This product is for clinical diagnosis and scientific research.

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