

**Product Catalogue Number FP231 For Research Use Only – RUO**

## NTRK1/NTRK2/NTRK3 Gene Break Apart Probe Detection Kit

**[Product Name]** NTRK1/NTRK2/NTRK3 Gene Break Apart Probe Detection Kit (Fluorescence In Situ Hybridization Method).

**[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.

**[Detection principle]**

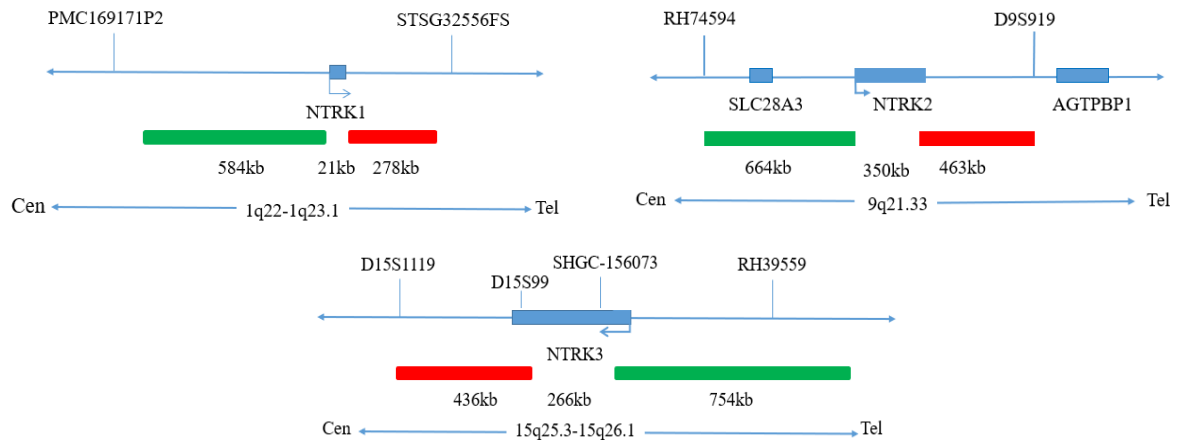
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Due to the fluorescence of the probe, the gene state of the hybrid probe and the target sequence can be clearly observed under the fluorescence microscope under the appropriate excitation light.

**[Product Main Components]**

The kit consists of one of NTRK1, NTRK2 or NTRK3 dual color probe as shown in Table 1.

**Table 1 Kit composition**

Component name	Cat.#	Specifications	Quantity	Main components
NTRK1dual color probe	FP231-1	100µL/Tube	1	NTRK1 Orange probe, NTRK1 Green probe
NTRK2dual color probe	FP-231-2	100µL/Tube	1	NTRK2 Orange probe, NTRK2 Green probe
NTRK3dual color probe	FP-231-3	100µL/Tube	1	NTRK3 Orange probe, NTRK3 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. The kit is transported below 0°C.

**[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 types: Paraffin-embedded specimens for surgical resection or biopsy.
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.

**[Testing Method]**

**1. Sample Pretreatment**

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd.'s "FISH Pretreatment Reagent " (Cat.# CL-003) for pretreatment.

**2. Denaturation and Hybridization**

The following operations should be performed in a 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.

**3. 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 for 1 min.
- ③ Remove and immerse the slides in a 0.3% NP-40/0.4xSSC 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.

**4. Counterstaining**

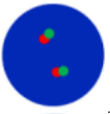
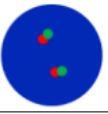
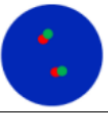

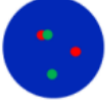
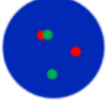
The following operations should be performed in a darkroom

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.

**5. FISH results observation**

Place the counterstained film under the fluorescence microscope, and first put it under the low-power objective lens (10x) Confirm the cell area under the microscope; Go to 40x under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100x) The fish results of nuclei were observed.

**[Common Signal Type Interpretation]**

<ul style="list-style-type: none"> <li>● NTRK1 gene site 3' signal</li> <li>● NTRK1 gene site 5' signal</li> </ul>	<ul style="list-style-type: none"> <li>● NTRK2 gene site 3' signal</li> <li>● NTRK2 gene site 5' signal</li> </ul>	<ul style="list-style-type: none"> <li>● NTRK3 gene site 3' signal</li> <li>● NTRK3 gene site 5' signal</li> </ul>
 <p style="text-align: center;"><b>Negative: 2 Fusion</b></p>	 <p style="text-align: center;"><b>Negative: 2 Fusion</b></p>	 <p style="text-align: center;"><b>Negative: 2 Fusion</b></p>
 <p style="text-align: center;"><b>Positive: 1 Orange 1 Green 1Fusion</b></p>	 <p style="text-align: center;"><b>Positive: 1 Orange 1 Green 1Fusion</b></p>	 <p style="text-align: center;"><b>Positive: 1 Orange 1 Green 1Fusion</b></p>

**[Limitations of test methods]**

- ① The results of this kit will be affected by various factors of the sample itself, but also limited by hybridization temperature and time, operating environment and the limitations of current molecular biology technology, which may lead to wrong results.
- ② Users must understand the potential errors and accuracy limitations that may exist in the detection process.

**[Precautions]**

1. This product is for research use only.
2. Please read this manual carefully before testing. The testing personnel should undergo professional technical training. The signal counter personnel must be able to observe and distinguish the orange-red and green signals.
3. The test will not provide any results when testing clinical samples it is difficult to count the hybridization signal and the sample is not enough to repeat the test, or the amount of cells is not enough for analysis.
4. The DAPI counterstaining agent used in this experiment is potentially toxic or carcinogenic. It must be operated in a fume hood. Inhalation and direct contact should be avoided by wearing the appropriate masks and gloves.
5. All chemicals are potentially dangerous. Avoid direct contact and wastes should be disposed off properly.

**[Manuscript version and approval date]**

Manual version: V1.2 reviewed on 07 December 2021

Approval date: 24 October 2019

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