

## NR4A3(9q22) Gene Break Apart Probe Detection Kit

**[Product Name]** NR4A3 (9q22) Gene Break Apart Probe Detection Kit (Fluorescence In Situ Hybridization Method).

### [Product Intended Use]

This kit performs in situ hybridization staining on the basis of conventional staining to provide physicians with auxiliary information for diagnosis. The test results are for clinical reference only and should not be used as the only basis for clinical diagnosis. Clinicians should make comprehensive judgment on the test results based on factors such as the patient's condition, drug indications, treatment response and other laboratory test indicators.

### [Detection Principle]

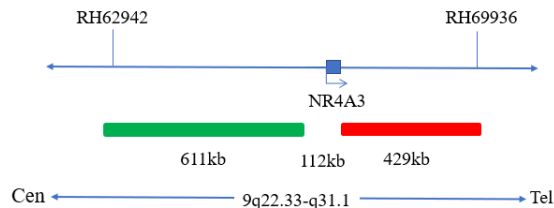
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of complementary base pairing, a specific probe is complementary to the target sequence in the cell. Because the probe is fluorescent, the hybridization probe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

### [Product Composition]

The kit consists of NR4A3 (9q22) dual color probe as shown in Table 1.

**Table 1: Kit composition**

Component name	Specifications	Quantity	Main components
NR4A3(9q22) dual color probe	100µL/Tube	1	NR4A3 Orange probe ; NR4A3 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 systems, including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

### [Sample Requirements]

1. Applicable specimens' types: Surgical resection or paraffin-embedded biopsy specimens.
2. Isolated tissue should be fixed in vitro with 4% neutral formaldehyde fixative within 1 hour. After tissue fixation, regular dehydration and paraffin embedding should be performed.

### [Instructions]

#### 1. Pretreatment

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd. pretreatment reagent kit (Cat# CL-003).

## 2. Denaturation & Hybridization

The following operations should be performed in a darkroom.

- ① Take the probe at room temperature for 5 minutes. Briefly centrifuge 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.

## 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 immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.
- ② Place the slides in a 2xSSC 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.

## 4. Dyeing

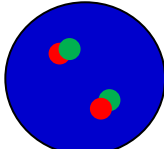
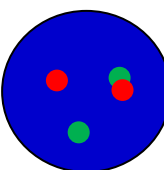
The following operations should be performed in a darkroom

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.

## 5. FISH results observation

Place the stained sections 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 (60x, 100x).

### [Common Signal Type Interpretation]

● NR4A3 signal	● NR4A3 signal
	<p><b>Negative:</b> 2 Fusions [Negative: 2F]</p>
	<p><b>Positive:</b> 1 Orange ; 2 Green ; 1 Fusion [Positive: 1R-1G-1F]</p>

NR4A3: Orange-red (R) pattern; NR4A3: Green (G) pattern

### Test Method Limitations

- 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 limitations of current molecular biology technology, which may lead to erroneous results.
- ② The user must understand the potential errors and accuracy limitations that may exist in the detection process.

**[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.

**[Manuscript version and approval date]**

Manual version: V1.0

Approval date: 16 January 2020

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