

NUT(15q14) Gene Break Apart Probe Detection Kit

(CW-253)

Intended use

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

Product composition

The kit consists of NUT dual-color probe (100 μ L/Tube).

Storage condition

Keep sealed away from light at $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$. 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 $2-8^{\circ}\text{C}$ in dark. For long-term preservation after opening, keep the lid sealed at $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$ 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.
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.

Pre-hybridization pretreatment

Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.

Dewaxing: According to the customer laboratory protocol (Commonly with Xylene for 15min).

Hydration: Take out the slides and put them respectively into 100%, 85% and 70% EtOH at room temperature for 3 minutes each.

Take out the slides, and immerse them in deionized water for 3 minutes. Remove the excess of water on the slides by air-drying. **Permeation:** Immerse the slides in deionized water at 100°C and boil continuously for 20-40 minutes (Conventional 20min). Remove the excess of water on the slides by air-drying.

Digestion: Protease enzymic digestion at 37°C for 10-40 minutes. Mix the protease work buffer (50mmol HCl) and the 10x protease solution (Pepsin concentration 5%) in a proportion of 9:1 to prepare the enzymatic digestion solution.

Washing: Wash with 2xSSC at room temperature for 5 minutes.

Dehydration: Take out the slides and dehydrate in 70%, 85%, and 100% gradient ethanol at room temperature for 2 minutes each time. Remove the excess of EtOH solution on the slides by air-drying.

Denaturation and hybridization

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

1. Take out the probe, let it stand at room temperature for 5min, turn it upside down with force, fully mix the probe, and then centrifuge briefly (vortex instrument oscillation is prohibited), take 10µL was dropped on the hybridization area of cell drops and immediately covered with 22mm×22mm cover glass, the probe shall be evenly expanded under the cover glass without bubbles, and the edge shall be sealed with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results during hybridization).
2. Put the tissue sections on the hybridizer, and denature at 85°C for 5min (the hybridizer should be preheated to 85°C in advance), and hybridize at 42°C for 2-16h.

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.

- 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.

FISH results observation

Place the stained slides under a fluorescence microscope and confirm the cells area under a low magnification objective (10×). Under magnification objective (40×) a uniform cells distribution is observed. Then the nuclei FISH results are observed under the high magnification objective (100×).

NUT gene site 3 signal NUT gene site 5 signal	
	Negative: 2 fusion
	Positive: 1 orange 1 green 1 fusion

Precautions

- 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.
- 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.
- 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.
- 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

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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]

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