

Product Cat. No.: GBS-174

For Research Use Only

NUP98 (11p15) gene break apart probe reagent Instructions Manual

[Product Name] NUP98 (11p15) gene break apart probe reagent

[Package Specifications] 10 Tests/box

[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]

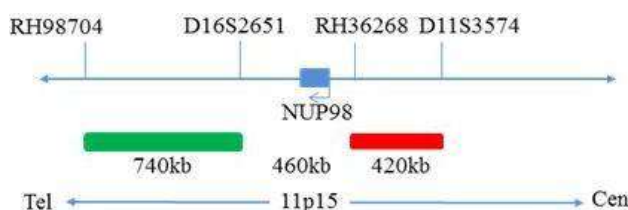
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Due to the fluorescence of the probe, the gene state of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

[Product Composition]

The kit consists of NUP98 dual-color probes, as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
NUP98 dual color probe	100μl/Tube	1	NUP98 Orange probe, NUP98 Green probe



[Storage conditions & Validity]

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. 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. Sample collection: take 1-3ml of bone marrow cells anticoagulated with heparin sodium
2. Sample preservation: unfixed fresh bone marrow specimen (stored at $2-8^{\circ}\text{C}$ for no more than 24 hours). after fixation, the cell suspension shall be stored at $-20 \pm 5^{\circ}\text{C}$ for no more than 12 months; The prepared cell slides can be stored at $-20 \pm 5^{\circ}\text{C}$ for no more than 1 month. When the sample storage temperature is too high or too low, or the cell suspension is volatilized excessively or polluted during storage, the sample will not be used for detection.

[Test method]

1. Sample processing before hybridization:

- ① Sample collection: Take 1-3mL of anticoagulated bone marrow cell samples.
- ② Cell harvesting: Place 3 mL of anticoagulated peripheral blood in a 15 mL centrifuge tube, centrifuge at 500g for 5 min, carefully discard the supernatant, and resuspend about 500μL of the residue.
- ③ Cell washing: Add 5 mL of 1×PBS buffer, mix and resuspend the cell pellet, centrifuge at 500g for 5 min, carefully discard the supernatant, and resuspend the cells with about 500μL of the residue; repeat 1 time.

- ④ Cells hypotonicity: Add 10mL of hypotonic solution pre-warmed to 37°C and place in an water bath at 37°C for 20min.
- ⑤ Cells pre-fixation: Pre-fix the cells by adding 1mL (10% by volume) of fixative solution to the cell suspension after the completion of hypotonic osmosis. Gently pipette, mix and centrifuge for 5 min at 500g, discard the supernatant, and resuspend about 500μL of the residue.
- ⑥ Cell fixation: Slowly add 10mL of fixative solution to the cell suspension at room temperature for 10 min, centrifuge at 500g for 5 min, and resuspend the cells with about 500μL of the residue; repeat once (the cells may be fixed several times until the cells pellet is washed and cleaned).
- ⑦ Cell suspension preparation: Pipet the supernatant and add the appropriate amount of fixative solution to prepare the appropriate cell suspension concentration.
- ⑧ Slides preparation: Pipet 3-5μl of cell suspension drop onto the slides, put at 56°C for 30min.
- ⑨ Pretreatment: At room temperature with 2×SSC (pH 7.0) solution, rinse the slide 2 times for 5min each time.
- ⑩ Dehydration: Place the slides in 70% ethanol, 85% ethanol and 100% ethanol for 2min each time, dehydrate and air dry.

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 88°C for 2 minutes (the hybridizer should be preheated to 88°C) and hybridize at 45°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.
- 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.

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 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×).

[Common Signal Type Interpretation]

<div> <div>● NUP98 gene site 5 signal</div> <div>● NUP98 gene site 3 signal</div> </div>	
	Negative : 2 fusion
	Positive : 1 orange 1 green 1 fusion

[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 only used for in vitro diagnosis.
2. Please read this manual carefully before testing. The testing personnel shall receive professional technical training, and the signal counting personnel must be able to observe and distinguish orange and green signals.
3. When testing clinical samples, when the hybridization signal counting is difficult and the sample is not enough to repeat the retest, or the cell volume is not enough for analysis, the test will not provide the test results.
4. DAPI counterstaining agent used in this experiment has potential toxicity or carcinogenicity, so it is necessary to operate in the fume hood, wear masks and gloves to avoid direct contact.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed of.

[Approval date and revision date of the instruction manual]

V1.0 approval date: April 10, 2020

V1.3 revision date: May 6, 2024