

## MALT1/IGH gene fusion t(14;18) probe reagent Instructions Manual

**[Product Name]** MALT1/IGH gene fusion t(14;18) 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]**

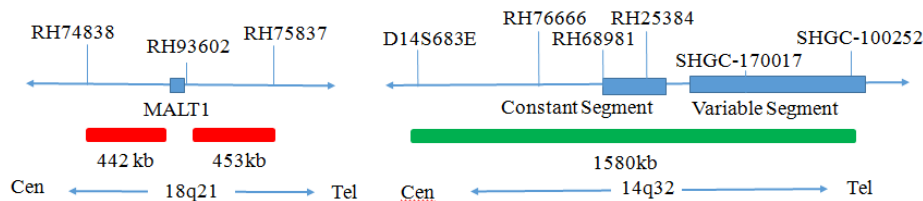
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Due to the fluorescence of the probe, the gene state of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

**[Product Main Components]**

The kit consists of MALT1/IGH dual color probe as shown in Table 1.

**Table 1 Kit composition**

Package Specifications	Component name	Specifications	Quantity	Main components
10 Tests/box	MALT1/IGH dual color probe	100µl/Tube	1	MALT1 Orange probe, IGH Green probe, hybridization buffer



**[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  $0^{\circ}\text{C}$ .

**[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 type: paraffin embedded specimen of surgical resection or biopsy tissue.
2. The tissue should be fixed with 4% neutral formaldehyde fixation solution within 1 hour after in vitro. After tissue fixation, it should be regularly dehydrated and embedded in paraffin.

**[Test method]**

### 1. Sample processing before hybridization:

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd. pretreatment reagent kit.

### 2. Denaturation and Hybridization

The following operations should be performed in a darkroom.

- ① Take out the probe put at room temperature for 5min. Mix and centrifuge briefly. 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 slides in the hybridization instrument, denature at  $85^{\circ}\text{C}$  for 5 minutes (the hybridizer should be preheated to  $85^{\circ}\text{C}$ ) and hybridize at  $42^{\circ}\text{C}$  for 2 to 16 hours.

**Cell sample:**

- ① Take out the probe put at room temperature for 5min. Mix and centrifuge briefly. 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 slides 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 immerse the slides in a 2XSSC solution for 5 seconds and remove the coverslip.
- ② Place the slides in a 2×SSC 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. Counterstaining**

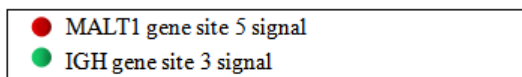
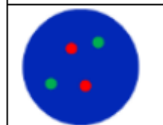
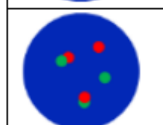
The following operations should be performed in a darkroom

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**

Put the counterstained cell drops under the fluorescence microscope, first under the low-power objective lens (10×) Confirm the cell area under the microscope; Go to 40× Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100×) .The FISH results of nuclei were observed.

**[Common Signal Type Interpretation]**

	
	Negative: 2 orange 2 green
	Positive : 1 orange 1 green 2 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 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.

**[Basic information]**

Name of registrant / manufacturer: Wuhan HealthCare Biotechnology Co., Ltd.

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**[medical device registration certificate No. / product technical requirement No.]**

Medical device registration certificate No.: ehxb20200283

**[approval date and modification date of the specification]**

V1.0 approval date: April 27, 2020

V1.3 approval date: December 7, 2021