

## **MALT1 Gene Break Apart Probe Detection Kit (CW-158)**

### **Intended use**

This kit uses orange fluorescein and green fluorescein to label the MALT1 probe. The MALT1 probe can be combined with the target detection site by in situ hybridization.

### **Product composition**

The kit consists of MALT1 dual color probe (100µL/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 includes fluorescence microscope and filter sets. The kit is labeled with orange fluorescein, and the filter set compatible with the fluorescent labeled dye should be selected.

DAPI: The maximum excitation wavelength is 367nm and the maximum emission wavelength is 452nm.

Orange fluorescence: The maximum excitation wavelength is 547nm and the maximum emission wavelength is 565nm.

Green fluorescence: The maximum excitation wavelength is 495nm and the maximum emission wavelength is 517nm.

### **Sample requirements**

#### ***Tissue samples:***

1. Applicable specimen type: surgical resection or paraffin embedded specimen of biopsy tissue.
2. The tissue should be fixed with 4% neutral formaldehyde fixative within 1 hour after the tissue is detached. After the tissue is fixed, it is often dehydrated and paraffin embedded.

**Cell samples:**

1. Applicable specimen type: fresh bone marrow specimen without fixation (stored at 2-8°C for no more than 24 hours).
2. Sample collection: take 1-3ml of heparin sodium anticoagulant bone marrow cells.
3. Sample preservation: after fixation, the cell suspension was stored at  $-20\pm 5^{\circ}\text{C}$  for no more than 12 months; the prepared cell slides could be stored at  $-20\pm 5^{\circ}\text{C}$  for no more than 1 month. When the storage temperature of the sample is too high or too low, when the cell suspension is too volatile or polluted, the sample cannot be used for detection.

**Related Reagents**

The following reagents are required for the experiment but not provided in this kit

**1. 20×SSC, pH 5.3±0.2**

Weigh 176g of sodium chloride and 88g of sodium citrate, dissolve in 800mL of deionized water, adjust the pH to  $5.3\pm 0.2$  at room temperature, and complete to 1 L with deionized water. High-pressure steam sterilization, stored at 2-8°C, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**2. 2×SSC, pH 7.0±0.2**

Take 100mL of the above 20xSSC, dilute with 800mL deionized water, mix, adjust the pH to  $7.0\pm 0.2$  at room temperature, complete to 1L with deionized water, stored at 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**3. Ethanol Solution: 70% ethanol, 85% ethanol**

Dilute 700ml, 850ml of ethanol with deionized water to 1L. The shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**4. 0.3% NP-40/0.4xSSC solution, pH 7.0-7.5**

Take 0.6mL NP-40 and 4mL 20×SSC, add 150mL deionized water, mix, adjust the pH to 7.0-7.5 at room temperature, with deionized water complete to a volume of 200mL. Stored at 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

**5. Fixation solution (methanol: glacial acetic acid = 3:1)**

Prepare a ready to use fixation solution by mixing thoroughly 30ml of methanol and 10ml of glacial acetic acid.

#### **6. 0.075M KCl solution**

Weigh 2.8g of potassium chloride, dissolve in 400mL of deionized water and complete to 500mL with deionized water. Stored at room temperature, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

#### **7. Diamidinyl phenylindole (DAPI) counterstain**

Use commercially available anti-quinching DAPI counterstain.

#### **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 500 $\mu$ L of the residue.
3. Cell washing: Add 5 mL of 1 $\times$ PBS buffer, mix and resuspend the cell pellet, centrifuge at 500g for 5 min, carefully discard the supernatant, and resuspend the cells with about 500 $\mu$ L 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 500 $\mu$ L 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 500 $\mu$ L 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 3-5 $\mu$ L of cell suspension drop onto the slides, put at 56°C for 30min.

#### **Denaturation and hybridization**

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

### ***Tissue samples:***

1. Take out the probe, leave it at room temperature for 5min, turn it upside down with force, mix it well, and then centrifuge it for a short time (vortex instrument vibration is prohibited). Take 10 $\mu$ l drop in the tissue hybridization area, and immediately cover the cover glass of 22mm $\times$ 22mm. The probe shall be evenly expanded under the cover glass without bubbles, and edge shall be sealed with rubber glue (the edge must be completely sealed to prevent dry chips from affecting the test results in the hybridization process).
2. The tissue sections were placed on the hybridizer and denatured at 78°C for 5min (the hybridizer should be preheated to 78°C in advance), and hybridized at 37°C for 16h.

### ***Cell samples:***

1. Take out the probe, leave it at room temperature for 5min, turn it upside down with force, mix it well, and then centrifuge it for a short time (no vortex instrument vibration). Take 10 $\mu$ l drop in the cell hybridization area, and immediately cover the cover glass of 22mm $\times$ 22mm. The probe should be evenly expanded under the cover glass without bubbles, and then seal the edge with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results in the hybridization process).
2. The cell drops were placed on the hybridizer and denatured at 85°C for 2 min (the hybridizer should be preheated to 85°C) and hybridized 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 $\times$ 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.

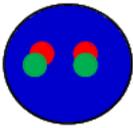
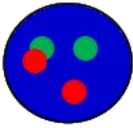
### **Counterstaining**

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 (10x). Under magnification objective (40x) a uniform cells distribution is observed. Then the nuclei FISH results are observed under the high magnification objective (100x).

● MALT1 gene 5' site signal	● MALT1 gene 3' site signal
	<b>Negative: 2 Fusions (2F)</b>
	<b>Positive: 1 Orange ; 1 Green ; 1 Fusion (1R ; 1G ; 1F)</b>

### 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]

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