API2/MALT1gene fusion probe reagent Instructions Manual

[Product Name] API2/MALT1gene fusion probe reagent

[Package Specifications] 10 Tests/box

[Intended Usage]

This kit performs fluorescence in situ hybridization staining based on 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.

[Detection Principle]

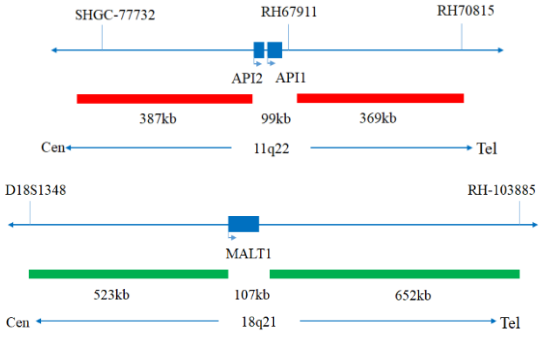
The kit is based on fluorescence in situ hybridization technology. The kit uses orange fluorescein-labeled API2 orange probe, and green fluorescein-labeled MALT1 green probe. The API2/MALT1 gene fusion probes can be bound to the target detection site by in situ hybridization technology. After the hybridization, the qualitative, quantitative, or relative positioning analysis of the gene to be measured under the microscope can be performed by the fluorescence detection system.

[Product Main Components]

The kit consists of USP6 dual-color probes, as shown in Table 1.

Table 1 Kit composition

Package Specifications	Component name	Specifications	Quantity	Main components
10 Tests/box	API2/MALT1 dual color probe	100μl/Tube	1	API2 orange probe, MALT1 green probe, hybridization buffer



[Storage conditions & Validity]

Keep sealed away from light at -20℃±5℃, and the validity period is 12 months.

After the cover is opened, it can be sealed and stored in 2~8℃ away from light within 24 hours. After the cover is opened, it should be sealed and stored in -20±5℃ away from light for a long time. Transport with temperature below 0℃.

[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]

Cell:

1. Take 1-3ml of heparin sodium anticoagulant bone marrow cells.

2. Sample preservation: Fresh bone marrow specimen without fixation (preserved at 2-8℃ for no more than 24 hours). After fixation, the cell suspension can be preserved at -20±5℃ for no more than 12 months; the prepared cell slide can be preserved at -20±5℃ for no more than 1 month. When the storage temperature of the sample is too high or too low, the cell suspension is volatilized excessively or polluted, the sample cannot be used for detection.

Tissue:

1. Applicable specimen types: Paraffin-embedded specimens from surgical excision or biopsy.

2. The tissue should be fixed with 4% neutral formaldehyde solution within 1 hour after isolation. After tissue fixation, it is routinely dehydrated

and embedded in paraffin.

[Related reagents]

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±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±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-quenching DAPI counterstain.

[Instructions]

Cells sample:

1. Sample processing before hybridization

① Sample collection: Take 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 hypotonicty: Add 10mL of hypotonic solution pre-warmed to 37°C and place in an water bath at 37°C for 15-20min.

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, rinse the glass slides twice with 2xSSC (pH 7.0) solution for 5min each time..

⑩ Dehydration: Place the glass slides in 70% ethanol, 85% ethanol and 100% ethanol and dry for 2 minutes.

Tissue sample:

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd.'s "FISH Pretreatment Reagent " (Cat# CL-003) for pretreatment.

2. Denaturation and Hybridization

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

Cell sample:

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 10μL of it and drop it into the cell drop hybridization area, immediately cover the cover glass of 22mm × 22mm. The probe should be evenly expanded under the cover glass without bubbles, and 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 88°C for 2min (the hybridizer should be preheated to 88°C) and hybridized at 45°C for 2 to 16 hours.

Tissue samples:

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 10 μ L was dropped on the hybridization area of cell drops and immediately covered with 22mm × For the 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, CO denature at 85 ℃ for 5min (the hybridizer should be preheated to 85 ℃ in advance), and hybridize at 42 ℃ for 2-16h.

3. Washing

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

① Carefully remove the sealing glue around the cover glass with tweezers to avoid sticking or moving the cover glass, immerse the sample in 2xSSC for about 5S, take it out, gently push a corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers;

② Place the sample at 2xSSC room temperature for 1 min;

③ Take out the sample and immerse it in 0.3%NP-40/0.4xSSC solution preheated at 68°C for 2min;

④ Take out the sample and immerse it in deionized water preheated at 37°C in advance for 1min; dry it naturally in the dark environment.

5. Counterstaining

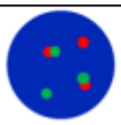
The following operations should be performed in a darkroom

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.

6. FISH results observation

Place the counterstained film under the fluorescence microscope, and first put it 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.

[Positive judgment value or reference interval]

API2 gene site signal MALT1 gene site signal	
	Negative :2 Orange, 2 Green
	Positive: 1 Orange 1 Green 2 Fusion

[Precautions]

① This product is only used for in vitro diagnosis.

② 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.

③ Users must understand the potential errors and accuracy limitations that may exist in the detection process.

④ 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.

Address: Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.

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Name of after-sales service unit: Wuhan HealthCare Biotechnology Co., Ltd.

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[approval date and modification date of the specification]

V1. 0 approval date: September 11, 2020

V1. 2 approval date: December 7, 2021