Product Cat. No.: FP-189 *For Professional Use*

ABL2 (1q25) gene break apart probe reagent Instructions Manual

[Product Name] ABL2 (1q25) gene break apart 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]

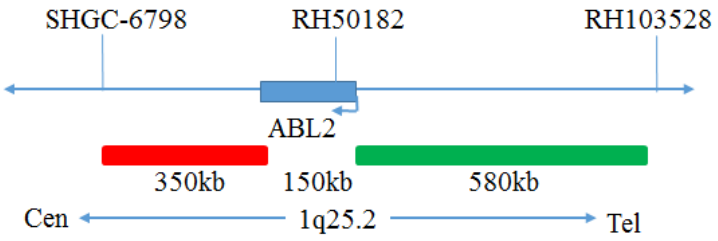
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of complementary base pairing, a specific probe binds complementary to the target sequence in the cell. Since the probe carries fluorescein, the hybridization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

[Product Composition]

The kit consists of ABL2 dual color probe as shown in Table 1.

Table 1 Kit composition

Package Specifications	Component name	Specifications	Quantity	Main components
10 Tests/box	ABL2 dual color probe	100μl/Tube	1	ABL2 orange probe, ABL2 green probe, hybridization buffer



[Storage conditions & Validity]

Keep sealed away from light at -20ºC±5ºC. 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 2-8ºC in dark. For long-term preservation after opening, keep the lid sealed at -20ºC±5ºC away from light. The kit is transported below 0ºC.

[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 sample type: unfixed fresh bone marrow samples (stored at 2-8°C for no more than 24 hours).
2. Sample collection take 1-3ml of bone marrow cells anticoagulated with heparin sodium.
3. Sample preservation: after fixation, the cell suspension shall be stored at -20±5°C for no more than 12 months; The prepared cell slides can be stored at -20±5°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.

[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±0.2 at room

temperature, and complete to 1 L with deionized water. High-pressure steam sterilization, stored at 2-8ºC, 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 2-8ºC, 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 2-8ºC, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑤Fixation 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]

1. Sample collection and slides preparation

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 300 g 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 300 g 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 37ºC and place in an water bath at 37ºC for 10-15min.

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 300 g, 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 300 g 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 56ºC for 30min.

2. Slides pretreatment

① At room temperature, rinse the glass slides twice with SSC (pH 7.0) solution for 5min each time.

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

3. 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 88°C for 2 minutes (the hybridizer should be preheated to 88°C) and hybridize at 45°C for 2 to 16 hours.

4. 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.

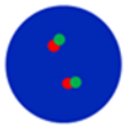
5. Counterstaining

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 slide under a fluorescence microscope. First confirm the cell area under a low magnification objective (10×); turn to a 40× objective lens to find an area where cells are evenly distributed; and finally observe the FISH results of nuclei under a high magnification objective lens (100×).

ABL2 gene site 5' signal ABL2 gene site 3' signal	
	Negative :2 Fusion
	Positive: 1 Orange 1 Green 1 Fusion

[Precautions]

① This product cannot be used for clinical diagnosis, but only for scientific research.

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.

Contact: 027-87570662

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

Contact: 18140559890

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[Medical device registration certificate No. / product technical requirement No.]

Medical device registration certificate No.: ehxb20190905

[Approval date and modification date of the specification]

V1. 0 approval date: October 24, 2019

V1. 2 approval date: December 7, 2021