

CBFB/MYH11 Gene Fusion Probe Detection Kit (CW-028)

Intended use

This kit uses Orange fluorescein to label CBFB, Green fluorescein to label MYH11 probe and bind EVI1 gene with the target site by in situ hybridization.

Product composition

The kit consists of CBFB/MYH11 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 20 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 $2\sim 8^{\circ}\text{C}$ 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 under 0°C .

Applicable instruments

Fluorescence microscope imaging system, including fluorescence microscope and filter set suitable for DAPI (367/452), green (495/517) and orange (547/565).

Sample requirements

1. Applicable specimen type: unfixed fresh bone marrow specimen (stored at $2\sim 8^{\circ}\text{C}$ for no more than 24 hours).
2. Sample collection: Take 1-3 mL of heparin sodium anticoagulated bone marrow cell sample.
3. Sample storage: After fixation, the cell suspension can 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 storage temperature of the specimen is too high or too low, or when the cell suspension is excessively volatile or contaminated during storage, the sample should not be used for testing.

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 2-8°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-queenching DAPI counterstain.

Sample collection and slide preparation

1. Sample collection: Take 1-3 mL of heparin sodium anticoagulated bone marrow cell sample.

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2. Cell harvest: Pipet uncultured bone marrow cells or cultured bone marrow cell samples into a 15mL conical centrifuge tube, centrifuge at 500g for 5 minutes, carefully aspirate and discard the supernatant, and leave about 500 μ L of residual liquid to resuspend the cells.
3. Cell washing: Add 5mL of 1 \times PBS solution by pipetting to mix and resuspend the cell pellet, centrifuge at 500g for 5min, carefully aspirate and discard the supernatant, keep about 500 μ L of residual liquid to resuspend the cells; repeat once.
4. Cell permeation: Add 10mL hypotonic solution to each tube (pre-warmed at 37 $^{\circ}$ C bath) and place at 37 $^{\circ}$ C water bath hypotonic for 20min.
5. Cell pre-fixation: Add 1mL (10% volume) of fixative to the cell suspension after permeation to pre-fix the cells, gently pipette to mix, and immediately centrifuge at 500g for 5min, and remove the supernatant, keep about 500 μ L of residual liquid to resuspend the cells.
6. Cell fixation: Slowly add 10 mL of fixative to the cell suspension, put at room temperature for 10 min to fix the cells. Centrifuge at 500g for 5 min, and keep about 500 μ L of residual liquid to resuspend the cells; repeat once (the cells can also be fixed multiple times until the cells precipitate and wash out).
7. Preparation of cell suspension: After the last cell fixation and centrifugation, aspirate the supernatant and add an appropriate amount of fixative to prepare the cell suspension with the appropriate concentration.
8. Producer: 3-10 μ L cell suspension was dropped onto the slide and aged at 56 $^{\circ}$ C for 0.5h.

Slide pretreatment process

1. Pretreatment: the slides were rinsed twice in 2 \times SSC solution at room temperature for 5min each time.
2. Dehydration: the cell drops were placed in 70% ethanol, 85% ethanol and 100% ethanol for 2 minutes respectively and then dried naturally.

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 10 μ l droplet in the cell and drop in the hybridization zone, immediately cover 22mm \times 22mm 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).

- 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.

Washing

The following operations should be performed in a darkroom.

- 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.
- Place the slides in a 2xSSC at room temperature for 1 min.
- Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution and wash for 2min.
- Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides 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 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.

	
	Negative :2 Orange 2 Green
	Positive: 1 Orange 1 Green 2 Fusion

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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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