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Your authentic partner in molecular cytogenetics

# BCL11B Gene Break Apart Probe Reagent

# (CW-425)

## Intended use

This kit uses Orange fluorescein to label the BCL11B orange probe and Green fluorescein to label the BCL11B green probe, and the BCL11B dual-color probe can be bound to the target detection site by fluorescence in situ hybridization.

## Product composition

The kit consists of BCL11B dual-color probe (100μL/Tube).

## Storage condition

Keep sealed away from light at -20°C±5°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°C in dark. For long-term preservation after opening, keep the lid sealed at -20°C±5°C away from light.

## 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: unfixed fresh bone marrow specimen (stored at 2-8°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±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 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.

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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.

**Sample processing before hybridization**

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 500μL of residual liquid to resuspend the cells.
3. Cell washing: Add 5mL of 1×PBS solution by pipetting to mix and resuspend the cell pellet, centrifuge at 500g for 5min, carefully aspirate and discard the supernatant, keep about 500μL of residual liquid to resuspend the cells; repeat once.
4. Cell permeation: Add 10mL hypotonic solution to each tube (pre-warmed at 37°C bath) and place at 37°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 500μL 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 500μL 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.

**Slide pretreatment procedure**

1. Pretreatment: the slides were rinsed twice in 2×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 or hybridization**

The following should be performed in a dark room.

1. Take the probe at room temperature for 5 minutes. Briefly centrifuge manually (do not use vortex or shaker instrument). 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).

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2. 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.

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 (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2). 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.

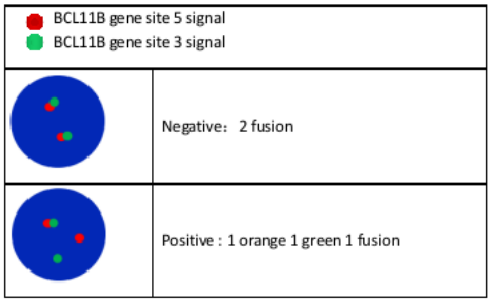
**Counterstaining**

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



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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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