

EPOR(19p13) Gene Break Apart Probe Detection Kit (CW-203)

Intended use

This kit performs in situ hybridization staining on the basis of conventional staining to provide physicians with auxiliary information for diagnosis. The test results are for clinical reference only and should not be used as the only basis for clinical diagnosis. Clinicians should make comprehensive judgment on the test results based on factors such as the patient's condition, drug indications, treatment response and other laboratory test indicators.

Product composition

The kit consists of EPOR (19p13) dual-color probe (100 μ L/Tube).

Storage condition

This kit is shipped below 0°C. 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°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 systems, including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

Sample requirements

1. Sample collection: Take 1-3 mL of heparin sodium anticoagulated bone marrow cell sample.
2. Sample storage: Unfixed fresh bone marrow cell specimen should be stored at 2-8°C for no more than 24 hours. After fixation, the cell suspension should be stored at $-20 \pm 5^{\circ}\text{C}$ for no more than 12 months. 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

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 pretreatment

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°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 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. Slides preparation: Pipette 3-10 μ L of the cell suspension onto the glass slide and bake at 56°C for 0.5h.
9. Pretreatment: Rinse the slides twice in 2 \times SSC solution at room temperature for 5 minutes each time.
10. Dehydration: Put the slides in 70%, 85% and 100% ethanol respectively for 2 minutes each time to dehydrate and then dry the slides naturally.

Denaturation and hybridization

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

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 μ 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).

- 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.

Washing

The following operations should be performed in a darkroom.

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

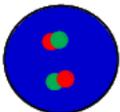
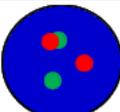
Counterstaining

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).

 EPOR signal	 EPOR signal
	Negative: 2 fusion Orange ; Green (Negative: 2F)
	Positive: 1 Orange ; 1 Green ; 1 Fusion [Positive: 1R ; 1G ; 1F]

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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