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Product Catalogue Number FP030 **For Research Use Only – RUO**

Chromosomes 4, 10 Centromere Probe Detection Kit

[Product Name]: Chromosomes 4, 10 Centromere Probe Detection Kit (Fluorescence In Situ Hybridization Method)

[Product Introduction]

The kit uses orange fluorescein-labeled 4q12 and CEP10 orange probe and green fluorescein-labeled CEP10 green probe to bind 4q12/CEP10 probe to the target detection site by in situ hybridization.

[Product Composition]

The kit consists of 4q12/CEP10 dual color probe as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
4q12/CEP10 dual color probe	100μL/Tube	1	4q12 Orange probe ; CEP10 Green probe

[Storage conditions & Validity]

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℃ in dark. For long-term preservation after opening, keep the lid sealed at -20°C± 5°C away from light. This kit shipment should be made below 0°C.

[Applicable Instruments]

Fluorescence microscopy 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 specimens can be stored at 4°C for no more than 24 hours; after fixation, bone marrow cell suspension can be stored at -20°C for no more than 6 months; the prepared bone marrow cell slides can be stored at -20°C for no more than 1 month.
2. When the specimen storage temperature is too high or too low (such as freezing), the sample will not be used for testing and should be discarded.
3. If the cell suspension is excessively volatilized or contaminated during storage, the sample should be discarded.

[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 in a graduated cylinder 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 Wuhan HealthCare Biotechnology supplied DAPI or the commercially available anti-quenching DAPI counterstain.

[Instructions]

1. Sample collection and slides preparation

① **Sample collection:** Take 3mL of heparin anticoagulated bone marrow samples.

② **Cell harvesting:** Aspirate marrow cells (uncultured or cultured) into a 15 mL conical centrifuge tube and centrifuge at 500g for 5 min. Carefully aspirate and discard the supernatant, leaving about 500µL of residual liquid to resuspend the cells.

③ **Cell washing:** Add 5mL of 1×PBS buffer, mix and resuspend the cell pellet, centrifuge at 500g for 5min, carefully discard the supernatant, and resuspend the cells with about 500µL of the residue; repeat one time.

④ **Cells hypotonicty:** Add 10mL of hypotonic solution pre-warmed to 37°C and place in a water bath at 37°C for 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 10min, centrifuge at 500g for 5min, 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.

2. Slides pretreatment

① At room temperature with 2×SSC solution (pH 7.0), rinse the slide 2 times for 5min each time.

② Place the slides in 70% ethanol, 85% ethanol and 100% ethanol for 2min each time, dehydrate and air dry.

③ Carry out the hhybridization experiment according to the hybridization procedure.

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. Dyeing

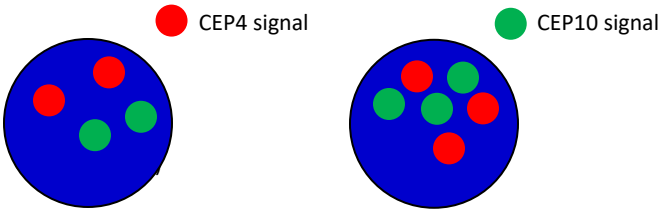
The following operations should be performed in a darkroom

Drop 10µl DAPI compound dye in the hybridization area of the glass slide and immediately cover. Select the suitable filter for glass slide observation under the fluorescence microscope.

6. FISH results observation

Place the stained sections under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10×); under magnification objective (40×) a uniform cells distribution is observed; then the nucleus size uniformity, nuclear boundary integrity, DAPI staining uniformity, no nuclei overlapping, cells clear signal are observed in the high magnification objective (60x, 100x).

[Interpretation of common signal types]



CEP4/CEP10 (Negative) CEP4/CEP10 (Positive)

[Precautions]

① This product is for research use only.

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 and waste should be properly disposed off.

[Manuscript version and approval date]
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