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Product Cat. No.: FP-005

For Professsional Use Only

RARA (17q21) probe reagent Instructions Manual

[Product Name] RARA (17q21) probe reagent

[Package Specifications] 10 Tests/box

[Product Introduction]

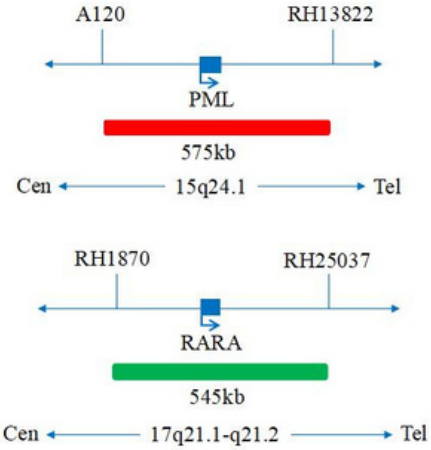
The kit uses orange fluorescein to label the PML orange probe and gzreen fluorescein to label the RARA green probe, and the PML/RARA probe can be bound to the target detection site by in situ hybridization.

[Product Content]

This kit consists of PML/RARA dual color probes as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
PML/RARA dual color probe	100μl/Tube	1	PML Orange probe, RARA Green probe



[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 under 0℃.

[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. Take 1-3ml of heparin sodium anticoagulant bone marrow cells.
2. Sample preservation: Fresh bone marrow specimen without fixation (preserved at 2-8ºC for no more than 24 hours). After fixation, the cell suspension can be preserved at -20±5ºC for no more than 12 months; the prepared cell slide can be preserved at -20±5ºC for no more than 1 month. When the storage temperature of the sample is too high or too low, the cell suspension is volatilized excessively or polluted, the sample cannot be used for detection.

[Test method]

Related reagents

① 20×SSC,pH5.3±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.

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.

[Instructions]

1. Sample collection and slide preparation

① Sample collection: take 1-3ml of heparin sodium anticoagulant bone marrow cells.

② Cell harvesting: the uncultured marrow cells or the cultured marrow cell samples were aspirated to a 15mL centrifuged tube at the bottom of the tip, and centrifuged at 500g for 5min. The supernatant was carefully aspirated and discarded, leaving about 500μL of residual liquid to suspend the cells again.

③ Cell washing: add 5ml of 1xPBS buffer solution, blow and mix up the heavy suspension cell precipitation, centrifugate 500g for 5min, carefully suck and discard the supernatant, and leave about 500μL of residual solution to heavy suspension cell; repeat once.

④ Cell hypotonic: add 10ml of hypotonic solution to each tube (37ºC warm bath in advance), and water bath at 37ºC hypotonic for 20min.

⑤ Cell pre fixation: add 1ml (10% volume) of fixed solution to the cell suspension after hypotonic treatment, gently blow and mix, centrifugate 500g immediately for 5min, remove the supernatant, and leave about 500μL of residual solution for cell suspension.

⑥ Cell fixation: slowly add 10ml of the fixed solution to the cell suspension, leave it at room temperature for 10min to fix the cell, centrifugate 500g for 5min, and leave about 500μL of the residual solution to re suspend the cell; repeat once (or fix the cell several times until the cell is precipitated, washed and cleaned).

⑦ Preparation of cell suspension: after the last centrifugation of cell fixation, the supernatant is sucked off, and a proper amount of fixed solution is added to make cell suspension with appropriate concentration.

⑧ Preparation: take 3-10μL cell suspension drop to slide, aging at 56ºC for 0.5h.

2. Slides pretreatment

① The slides were rinsed twice in 2xSSC 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.

3. 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 10μL of it and drop it into the cell drop hybridization area, immediately cover the cover glass of 22mm × 22mm. The probe should be evenly expanded under the cover glass without bubbles, and 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).

② The cell drops were placed on the hybridizer and denatured at 88ºC for 2min (the hybridizer should be preheated to 88ºC) and hybridized at 45ºC for 2 to 16 hours.

4. Washing

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

① Carefully remove the sealing glue around the cover glass with tweezers to avoid sticking or moving the cover glass, immerse the sample in 2xSSC for about 5S, take it out, gently push a corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers;

② Place the sample at 2xSSC room temperature for 1 min;

③ Take out the sample and immerse it in 0.3%NP-40/0.4xSSC solution preheated at 68ºC for 2min; (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).

④ Take out the sample and immerse it in deionized water preheated at 37°C in advance for 1min; dry it naturally in the dark environment.

5. Counterstaining

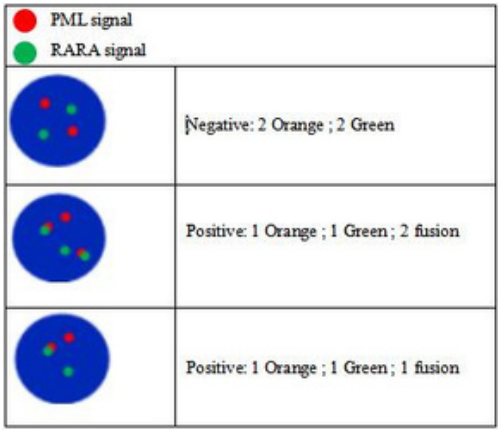
The following operations should be performed in a darkroom

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 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.

[Common Signal Type Interpretation]



[Precautions]

① 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.

② Users must understand the potential errors and accuracy limitations that may exist in the detection process.

③ All chemicals are potentially dangerous. Avoid direct contact. Used kits are hazardous wastes and should be properly disposed of.

[Basic information]

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Manufacturer

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