4448 Ammendale Rd Beltsville, MD 20705, USA
Tel# +1 (408) 580-1396
E-mail: info@genebiosolution.com
Website: www.genebiosolution.com

Product Cat. No.: GBS-043

For Research Use Only.

RARA gene break apart probe reagent Instructions Manual

[Product Name] RARA gene break apart probe reagent

[Package specification] 10 Tests /box

[Product Introduction]

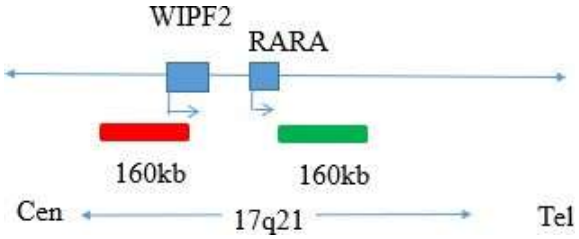
This kit uses orange fluorescein and green fluorescein to label the RARA probe. The RARA probe can be combined with the target detection site by in situ hybridization.

[Product Main Components]

The kit consists of RARA dual color probe as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
RARA dual color probe	100μL/Tube	1	RARA orange probe ; RARA green probe



[Storage conditions & Validity]

Keep sealed away from light at -20℃±5℃. 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℃±5℃ away from light.

[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: fresh bone marrow specimen without fixation (stored at 2-8℃ for no more than 24 hours).

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

3. Sample preservation: after fixation, the cell suspension was stored at -20±5℃ for no more than 12 months; the prepared cell slides could be stored at -20±5 ℃ for no more than 1 month. When the storage temperature of the sample is too high or too low, when the cell suspension is too volatile or polluted, the sample cannot be used for detection.

4448 Ammendale Rd Beltsville, MD 20705, USA
Tel# +1 (408) 580-1396
E-mail: info@genebiosolution.com
Website: www.genebiosolution.com

[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 1L with deionized water. High-pressure steam sterilization, stored at 2-8℃, 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℃, 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 pretreatment of hybridization

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

② Cell harvesting: suck the uncultured bone marrow cells or the cultured bone marrow cell samples into a 15ml tip bottom centrifuge tube, centrifugate 500g for 5min, carefully suck and discard the supernatant, and leave about 500µll residual liquid to suspend the cells again.

③ Cell washing: add 5ml of 1×PBS 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 (37℃ warm bath in advance), and water bath at 37℃ 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 56℃ for 0.5h.

4448 Ammendale Rd Beltsville, MD 20705, USA
Tel# +1 (408) 580-1396
E-mail: info@genebiosolution.com
Website: www.genebiosolution.com

2. 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.

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

Cell samples:

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 drop in the cell hybridization area, and immediately cover the cover glass of 22mm×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).

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

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 2×SSC 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 68 ℃ for 2min;

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

5. Counterstaining

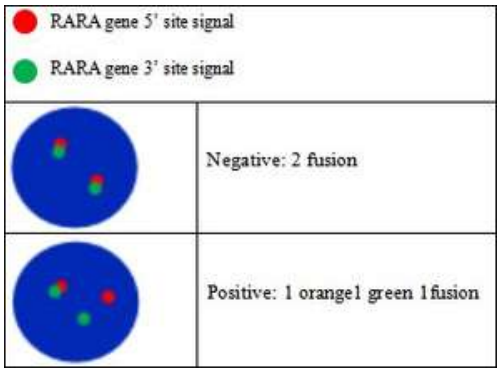
The following operations should be performed in a darkroom

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 stained sections under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10x); under magnification objective (40x) 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 (100x).

4448 Ammendale Rd Beltsville, MD 20705, USA
Tel# +1 (408) 580-1396
E-mail: info@genebiosolution.com
Website: www.genebiosolution.com

[Interpretation of common signal types]



[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.

③All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed off.