Cytowish Ltd
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Your authentic partner in molecular cytogenetics

FGFR3 Gene Break Apart Probe Detection Kit

(CW-112)

Intended use

This kit uses orange fluorescein and green fluorescein to label the FGFR3 probe. The FGFR3 probe can be combined with the target detection site by in situ hybridization.

Product composition

The kit consists of CEP7 green mono-color probe (100μL/Tube).

Storage condition

The kit is transported below 0°C. 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

Tissue sample

1. Applicable specimen type: fresh bone marrow specimen without fixation (stored at 2-8°C 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°C for no more than 12 months; the prepared cell slides could be stored at -20±5°C 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.

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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-quenching DAPI counterstain.

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Sample pretreatment

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°C warm bath in advance), and water bath at 37°C 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°C for 0.5h.

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 and hybridization

Tissue sample

The following operations should be performed in a darkroom.

Cell samples:

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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°C for 2 min (the hybridizer should be preheated to 88°C) and hybridized at 45°C for 2-16h.

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 place the slides into 2xSSC for 5 seconds, and gently remove the coverslip.
2. Place the glass slides in 2xSSC at room temperature for 1 min.
3. Remove and immerse the slides in a 0.3% NP-40/0.4×SSC solution preheated at 68°C for 2 min.
4. 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 (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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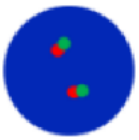
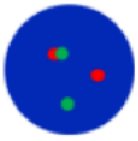
FGFR3 gene site 3 signal FGFR3 gene site 5 signal	
	Negative: 2 fusion
	Positive : 1 orange 1 green 1 fusion

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]

Manual version: V1.0

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