

13q Gene Probe Detection Kit (CW-305-1)

Intended use

The kit uses orange fluorescein-labeled RB1(13q14) probe and green fluorescein-labeled 13q34(LAMP1) probe to bind the MLL probe to the target detection site by in situ hybridization.

Product composition

The kit consists of RB1/13q34 dual-color probe (100 μ L/Tube).

Storage condition

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 $2-8^{\circ}\text{C}$ 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 system including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

Sample requirements

1. Applicable specimen type: unfixed fresh bone marrow specimens shall be stored at 4°C for no more than 24 hours; After fixation, the bone marrow cell suspension was 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 storage temperature of the sample 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 volatilized excessively or polluted during storage, the sample shall be discarded.

Related reagents

1. 20 \times SSC, pH 5.3 \pm 0.2

Weigh 176g of sodium chloride and 88g of sodium citrate, dissolve in 800mL of deionized water, adjust the pH to 5.3 \pm 0.2 at room temperature, and complete to 1 L with deionized

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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 20×SSC, 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-40/0.4×SSC 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.

Pretreatment

1. Sample collection: Take 3mL anticoagulated bone marrow cell samples.
2. Cell harvest: The bone marrow cells sample is pipetted to the tip of centrifuge tube and centrifuge at 1000rpm for 10 minutes to remove the supernatant. Place 3mL of anticoagulated peripheral blood in 15 mL centrifuge tube and centrifuge at 300g for 5min. Carefully aspirate the supernatant and resuspend the cells with approximately 500µL of residue.

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3. Cell washing: Add 5mL of 1×PBS buffer, mix and resuspend the cell pellet, centrifuge at 300g for 5min, carefully discard the supernatant, and resuspend the cells with about 500µL of the residue; repeat 1 time.
4. Cells hypotonicity: Add 10mL of hypotonic solution pre-warmed to 37°C to each tube and put at 37°C for 15-20min in the hypotonic water bath.
5. Cell Pre-fixation: Pre-fixed cells are added to the cell suspension after addition of 1 mL (10% by volume) fixative solution. Gently mix and immediately centrifuge at 300g for 5min, remove the supernatant, and resuspend the cells with 500µL of residual solution.
6. Fixation: Slowly add 10 mL of fixative solution to the cell suspension, fix the cells at room temperature for 10 min, centrifuge at 300g for 5 min, and resuspend the cells with about 500 µL of the residue; repeat once (the cells may be fixed several times until the cells are washed and cleaned).
7. Cell suspension preparation: Pipet the supernatant and add the appropriate amount of fixative solution to prepare the appropriate cells suspension concentration.
8. Slides preparation: Pipet 3-5µL of cell suspension drop onto the slides, put at 56°C for 30min.

Slides preparation

1. At room temperature with 2×SSC (pH 7.0) solution, 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.

Denaturation and hybridization

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

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.
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).

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.

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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: [V1.2 reviewed on 07 December 2021](#)

Approval date: [15 April 2020](#)