Cytowish Ltd
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Your authentic partner in molecular cytogenetics

20q12 (D20S108/CEP8) Probe Detection Kit

(CW-011-5)

Intended use

The kit uses orange fluorescein-labeled D20S108 orange-red probe and green fluorescein-labeled CEP8 green probe to bind D20S108/CEP8 probe to the target detection site by in situ hybridization.

Product composition

The kit consists of D20S108/CEP8 dual color probe (100 μl/Tube).

Storage condition

Keep sealed away from light at -20oC±5°C. The product is valid for 20 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. The kit is transported under 0°C.

Applicable instruments

Fluorescence microscopy imaging system including fluorescence microscopy and filter sets suitable for DAPI, Green, and Orange.

Sample requirements

1. Applicable specimen types: Fresh bone marrow specimens stored at 4°C for less than 24 hours and used for FISH detection after culture.

2. When specimens are stored at too high or too low a temperature (eg, frozen), the specimen will not be used for testing and should be discarded.

3. Bone marrow cell suspensions for karyotype analysis should be stored at -20°C for FISH detection.

4. If the cell suspension is excessively volatile or contaminated during storage, the sample should be discarded.

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Test method

Related Reagents

The following reagents are required for the experiment but not provided in this kit:

1. 20×SSC (sodium citrate buffer), 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. 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.

4. Fixative 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.

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.

5. PBS buffer, pH 7.4±0.2

Prepare a ready to use fixation solution by mixing thoroughly 30ml of methanol and 10ml of glacial acetic acid.

Components: Sodium chloride (8g), Potassium chloride (0.2g), Sodium hydrogen phosphate (3.58g), Potassium dihydrogen phosphate (0.27g).

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Dissolve the above reagents in 800mL deionized water, mix, adjust the pH to 7.4 ± 0.2 at room temperature, with deionized water complete to a volume of 1000mL. Stored at room temperature for 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

6. 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.

7. 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.

8. HCl Solution

Measure 8.2ml concentrated HCl, mix with deionized water and complete to 100ml, store at room temperature to obtain 1M HCl. Based on needs dilute to 0.01M by 10-fold dilution method.

9. Di-amiindyl phenyl indole (DAPI) dyeing agent

Please use commercially available DAPI counterstains containing anti-quencher.

Sample collection and slides preparation

1. Sample collection: Take 3mL of heparin anticoagulated bone marrow samples.
2. Cell harvesting: The bone marrow cells sample (cultured or uncultured) is pipetted in to a 15mL tip of centrifuge tube and centrifuge at 500g for 5 minutes to remove the supernatant.
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.

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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.
9. **Slides pretreatment**

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

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.

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.

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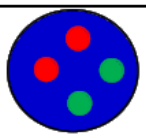
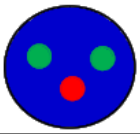
Counterstaining

The following operations should be performed in a darkroom.

Drop 10-15μl DAPI compound dye in the hybridization area of the glass slide and immediately covered for 10-20min. Select the suitable filter for glass slide observation under the fluorescence microscope.

FISH results observation

Place the counterstained glass slide under the fluorescence microscope and under the natural light, first under the low power objective lens (10x) to confirm the cell area under the microscope; Go to 40× Under the objective lens, find a position where the cells are evenly distributed; At high power objective (60x, 100x) select cells with complete nuclear boundary, uniform DAPI staining, no overlapping nuclei and clear signals. At least 200 cells should be randomly selected to count the orange, green and yellow signals in the nucleus.

D20S108 gene site signal	CEP8 gene site signal
	**Negative**: 2 Orange and 2 Green (2R ; 2G)
	**Positive**:1 Orange and 2 Green (1R ; 2G)

Test method limitations

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 limitations of current molecular biology technology, which may lead to erroneous results.
2. The user must understand the potential errors and accuracy limitations that may exist in the detection process.

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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: V1.1 reviewed on 20 November 2021

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