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Your authentic partner in molecular cytogenetics

MDS Chromosome and Gene Anomaly Probe Detection Kit

(CW-011-6)

Intended use

This kit uses fluorescence in situ hybridization to detect the absence/deletion of chromosome Y chromosomal anomalies in patients with myelodysplastic syndrome. The test sample is bone marrow cells.

Myelodysplastic syndrome (MDS) is a group of heterogeneous diseases that are generally considered to originate from hematopoietic stem cells and belong to malignant clonal diseases. Studies have shown that 40% to 60% of patients with MDS have non-randomized chromosomal anomalies, of which - the most common are 5/5q-, -7/7q-, +8, 20q-, and -Y. Among the common chromosomal anomalies' in patients with MDS, some chromosomal anomalies have specific diagnostic value. Immunosuppressive therapy is effective in some patients with simple +8, 20q-, or Y-; karyotype analysis also has important value in the classification, treatment, and prognosis of MDS, such as single Y-, 5q- or 20q- patients' have a good prognosis, while patients with complex chromosomal anomalies (≥3 anomalies) or chromosome 7 anomalies have a poorer prognosis. Patients with other anomalies have a moderate prognosis. These anomalies are of great significance in the diagnosis, treatment, and prognosis of MDS.

This kit was not clinically combined with gene targeting therapy, and was validated only for the performance of gene detection. This kit is only suitable for the detection of myelodysplastic syndromes and provides physicians with supplementary diagnostic information.

Product composition

This kit consists of CEPY/CEPX hybridization 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.

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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.

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.

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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)

Fill the flask with 30mL of methanol and 10mL of glacial acetic acid and mix thoroughly for immediate use.

5. PBS buffer, pH 7.4±0.2

Take Sodium Chloride (8g), Potassium Chloride (0.2g), Monosodium Hydrogen Phosphate (3.58g), Potassium Dihydrogen Phosphate (0.27g). Dissolve the aforementioned components in 800mL of deionized water, adjust the pH to 7.4±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.

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 uncultured or cultured bone marrow cells sample is pipetted to the tip of centrifuge tube and centrifuge at 1000rpm for 10 minutes to remove the supernatant.

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3. Low permeability: Wash cells once with PBS, centrifuge at 1000rpm for 10 minutes, and pipet the supernatant. Add 0.075mol/L KCL solution (6~8mL) pre-warmed at 37°C, mix with a pipette, and store in an incubator at 37°C for 20 to 30 minutes.
4. Pre-fixation: Add 2mL of 3:1 methanol, glacial acetic acid fixative solution and mix evenly. Centrifuge at 1000rpm for 10min.
5. Fixation: Aspirate the supernatant; add freshly prepared 5mL of 3:1 methanol - glacial acetic acid fixative solution, mix evenly, fix for 10 min, centrifuge at 1000 rpm for 10 min.
6. Repeat step (5) twice.
7. Cells suspension preparation: Pipet the supernatant and add the appropriate amount of fixative solution to prepare the appropriate cells suspension concentration.
8. Slides preparation: Use a pipette to gently stir the cell suspension, then blot it off and place it onto a clean, fat-free glass slides soaked in ethanol. Drip 1 to 2 drops per slide, put at 56°C for 30min. The prepared slides can be stored in a refrigerator at 4°C or in a refrigerator at -20°C for about 1 to 4 weeks.

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.

Take out the prepared slide and mark it. Take out the CEPY/CEPX probe, briefly centrifuge after mixing. The probe sunk to the bottom of the tube, and take 10μL of each and drop on the seven slides hybridization zone. Immediately cover the 22mmx22mm glass slide area; spread evenly without bubbles the probe under the glass slides covered area and seal edges with rubber (edge sealing must be thorough to prevent dry film from affecting the test results during hybridization).

Place the glass slides in the hybridizer, denature at 88°C for 2 minutes (the hybridizer should be preheated to 88oC) and hybridize at 45°C for 2 to 16 hours.

Washing

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The following operations should be performed in a darkroom.

Use tweezers to carefully tear off the cover glue around the slides. Avoid sticking off or moving the cover glass. Immerse the slides in 2xSSC for about 5 seconds. Take out and gently push the cover glass to the edge of the slides with tweezers. Use tweezers to gently remove the cover slide.

Place the slides in 2xSSC at room temperature for 1 min.

Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution, wash for 2min.

Remove the slides and immerse in deionized water preheated at 37°C for 1 min. Dry the slides naturally in the dark.

Counterstaining

The following operations should be performed in a darkroom.

Dip 10µL of DAPI counterstain into the hybridization area of the glass slide, immediately cover, and then use the suitable filter to observe the sections 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 40x 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.

Positive value determination or reference interval

Signal classification and counting

1. Normal cells signal: There are one Orange-red signal and one Green signal in single interphase nuclei after CEPY/CEPX hybridization.
2. Anomaly signals: CEPY/CEPX there is only one Green signal in a single interphase nucleus, and the Y chromosome is missing.

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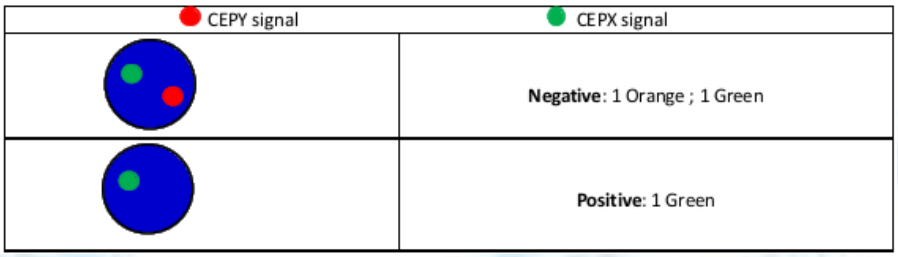
Randomly count 200 cells, and the number of normal signal cells and different abnormal signal cells are counted. Each cell is counted once and only cells with a hybridization signal (both color signals) are counted. No signal or only a single color signal is counted for CEPY/CEPX and weak or over dispersed cells are not counted.



FISH results determination

Abnormal results detection requires anomaly threshold establishment.

1. **Anomaly threshold**
   - It is recommended to select 20 patients with non-leukemia or normal human bone marrow samples as negative controls.
   - Use the above method to prepare the slides for FISH experiments.
   - Anomaly threshold setup: Each sample is analyzed for 200 cells. The percentage of abnormal signals in each probe group is counted.
     The mean and standard deviation of the percentage of cells showing abnormal signal patterns is calculated. The abnormal threshold is defined as mean +3 x standard deviation.

Abnormal Threshold = Mean (M) + 3 × Standard Deviation (SD)

Example: Table 2: 20 non-leukemia patients or normal subjects were selected as negative controls for FISH detection.

Abnormal threshold setup

No.	Abnormal cells (%)
Sample 1	5
Sample 2	3
....	....
Sample 20	4
Mean	3
SD	0.3
Threshold value	(Anomaly threshold = Mean value + 3 x SD) = 3.9

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Results determination

If the detection value of cells number showing abnormal signal is greater than the anomaly threshold, it is determined as a Positive result. If the detection value of cells number showing the abnormal signal is less than the anomaly threshold, it is determined as a Negative result. If the detection value of cells number showing abnormal signal is equal to the anomaly threshold, increase the counting to 500 cells the number of observations of the sample cells, to determine the result.

Test method limitations

This kit is for fresh bone marrow cells and is not recommended for use in other cells. Any change may alter the results of the test. The detection of chromosome Y cannot be used for the detection of a single base mutation, and cannot be used as the sole basis for the treatment, prognosis, or other clinical management of patients with myelodysplastic syndromes. A comprehensive assessment based on the patient's medical history and other diagnostic results is required.

Product Performance Index

1. Fluorescence signal strength: After the probe effective hybridization with the karyotype reference material, the probe should emit fluorescence signals which can be identified by the naked eye under the fluorescence microscope.

2. Sensitivity:

2.1 The sensitivity of CEPY probe was analyzed in 100 chromosome of chromosome Y in metaphase division of 50 cells, and at least 98 of chromosome Y showed 1 orange-red fluorescence signal.

2.2 The sensitivity of CEPX probe was analyzed in 100 chromosome of chromosome X in metaphase division of 50 cells, and at least 98 of chromosome X showed 1 green fluorescence signal.

3. Specificity:

3.1 The specificity of CEPY centromeric probe was analyzed in 100 chromosome of chromosome Y in metaphase division of 50 cells, and at least 98 of chromosome Y showed 1 specific orange-red fluorescence signal in the target area.

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3.2 The specificity of CEPX centromeric probe was analyzed in 100 chromosome of chromosome X in metaphase division of 50 cells, and at least 98 of chromosome X showed 1 specific green fluorescence signal in the target area.

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.

References

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