AML1/ETO Gene Fusion Kit（FISH）
Instruction For Use

[Product Name]

AML1/ETO Gene Fusion Kit（FISH）

[Specification]

5 tests/kit, 10 tests/kit, 20 tests/kit.

[Intended Use]

It was used to qualitatively test the gene fusion of AML1/ETO gene for acute myeloblastic leukemia (AML) patients.

1) Auxiliary diagnosis: AML1-ETO fusion gene mainly occurs in the M2 AML patients (about 40%), and is rare in M4, M1 and myelodysplastic syndromes (MDS). The positive rate of M2b is 90%. It can be used as an important molecular marker for the diagnosis of M2b typing.

2) Prognosis: The positive AML1-ETO fusion gene is a sign of good prognosis, the complete remission rate can reach 90%, and the 5-year long-term disease-free survival rate can reach 50%-70%. Among them, adult patients have good response to treatment with high complete remission rate and long median survival time, but are prone to relapse; the treatment and prognosis of children are not as good as those of adults.

[Test Principle]

Based on the principle of complementary base pairing, marked the fluorescent stain in DNA fragments (probe) matching with the target DNA.The probe and the corresponding DNA fragments in material waiting for test specificaly combined under certain conditions (hybrid), to form double-stranded nucleic acid, by means of fluorescence microscope to observe and record the hybrid double chain type, quantity, to judge target DNA in inspected samples normal or not.

The samples were fixed by 10% neutral formalin, paraffin-embedded and sliced, then placed on a slide. The denatured DNA formed a single strand state and hybridized with the probe. After hybridization, unbound probes are washed away. Use DAPI (4,6- diimiyl -2- diphenylindoles) to restain the nucleus. DAPI is a nucleic acid stain used as a specific DNA stain. The kit's probe hybridization signals can be observed by fluorescence microscopy equipped with corresponding filters (blue, red and green fluorescent signals).Fluorescence signals in the nucleus were observed under a microscope.

After hybridization, two ETO gene (green) and two AML1 (red) signals were counted. Otherwise, If the AML1/ETO region fuses, the red and green signals fuse into a yellow signal.

[Main Components]

The kit is mainly composed of AML1/ETO probe and DAPI counterstain solution.

The reagent does not contain but necessary for the test: FISH Sample Pretreatment Kit.

Other reagents not provided: positive contrast photo; Xylene; Ethanol (anhydrous, 85%, 70%);Purified water; Sealing gum.

[Storage and Validity]

Stored at -20℃±3℃ in dark, sealed conditions, valid for 12 months.

[Applicable Instrument]

Fluorescence in situ hybridization system;

Fluorescence microscope, in which:

Objective lens: It is recommended to use oil-immersed fluorescent objective lens with aperture ≥0.75.

Immersion Oil: Low level self-fluorescence Immersion Oil exclusive use for fluorescence microscopy.

Optical Filters: It is recommended to select filters suitable for labeled fluorescent stains.

Green fluorescence: excitation wavelength is 490nm , and emission wavelength was 516nm.

Orangered fluorescence: excitation wavelength was 551nm ,and emission wavelength was 572nm.

[Sample Requirements]

Collect specimens: bone marrow (2-3ml) or peripheral blood (3-5ml). Heparin sodium anticoagulation. The amount of specimens depends on the specific conditions of the patient such as aplastic anemia patients with less leukocytes should increase the collection.

Specimen storage and transportation: after collection, it should be processed within 2h. Stored at 2℃ ~ 8℃ within 72h.

[Test Method]

Specimen collection:

1. Take out 2~3ml peripheral blood or bone marrow (heparin sodium anticoagulation) and centrifuge at 2000rpm for 5 minutes, discard the supernatant.
2. Take 1ml cell, add 10ml of 0.075mol/L KCl, mix well and stand for 2 minutes.
3. Constant temperature water bath 37±1℃ hypotonic for 20 minutes.
4. Add 1ml stationary liquid, mix well, and pre-fix for 10min at room temperature.
5. Mix well and centrifuge at 2000rpm for 5min.
6. Discard the supernatant, after precipitation, add 10ml stationary liquid, mix well, and precipitate at -20℃ for 30min.
7. Centrifuge at 2000rpm for 5 minutes, discard the supernatant.
8. The above washing steps can be repeated until the cell precipitation is washed white and clean (repeated washing does not need to stand for 30min).

Slide preparation:

1. Take out a clean slide

2. After resuspending cells, take 3μl, drop it to a glass slide, and dry it at room temperature.

3. Observe the cell density under a phase contrast microscope with a 10× objective lens. It is required that the cells do not overlap, and the appropriate number of cells is 100-200 in a single field of view.

3.1 If the cell density and number are appropriate, proceed to step 3

3.2 If cells overlap, dilute the cell suspension by adding appropriate fresh stationary liquid.

3.3 If the cell density is low, centrifuge at 2000 rpm for 5min, carefully aspirate an appropriate amount of supernatant, and after mixing, take out 3μl to prepare slide, dry it and observe.

4. Observe under a phase contrast microscope. If there is too much cell debris, pretreatment is required and an appropriate hybridization area is selected.

Note: At least one additional slide should be prepared for each case, it should be placed in a closed container with absolute ethanol, and can be stored for 12 months at -20℃±3. The remaining cell suspension can be stored at 2-8℃ for one month, and can be prepared again if necessary.

5. Bake specimen slide at about 65°C for more than 3h.

Slide Pretreatment:

1. Soak in the 2×SSC solution at room temperature for 2min.

2. Dehydration: the slides were successively treated in 70%, 90% and anhydrous ethanol solution for 2 min.

3. Remove the slide and then dried at room temperature.

Denaturation and hybridization (avoid light):

1. Remove the probe,mix and centrifuge. Take 10 μ L probe and drop it on the target area. Cover the coverslip and avoid air bubbles.

2. Seal it with the sealing gum. Note that the coverslips are completely prevented from volatilizing the hybridization solution.

3. Put the slides into a hybridization instrument, denature at 83℃ for 6min, and hybridize at 40℃ for 18h.

Washing (avoid light):

1. Put the washing buffer I into the water bath and heat it to 72℃.

2. Gently remove the sealing gum from the slide with tweezers. Soak the glass slides in the washing buffer II solution at room temperature, placed for about 2 minutes, and remove the coverslip.

3. Immerse the slides again in 72℃ washing buffer I for 2min.

4. Remove the glass slides in the washing buffer II solution at room temperature, placed for about 2 minutes

5. wash the slides in 70% and 85% ethanol solution for 2min in turn and dry them

6. Add 10 μ L DAPI to the target area of the slide. Avoid air bubbles and cover with a coverslip, incubate for 10-20min at -20℃.

7. Microscope examination: The slides were placed in -20℃ storage condition and then taken out for observation and analysis under a fluorescence microscope.

[Analysis of FISH test results]

200 cells are counted, and count the number of positive cells. Common type of abnormality: Red and green signals fuse to form a yellow signal.

Normal cells: The type of signal in the cell is represented by two red signals (2R) and two green signals (2G).

Abnormal cells: The type of intracellular signal is reflected in the fusion of red and green signals to form a yellow signal in a single cell.

[Attentions in result analysis]

1. Tumor cells should be randomly counted in the samples.

2. Counting cells must have clear and recognizable signals in each channel.

3. Do not analyze the heterogeneous regions.

4. Do not analyze nucleus with unclear outline or overlap.

5. Do not analyze the area where the background is so deep that affects the signal judgment.

6. Counting results shall be independently completed by two participants, and the results should be consistent before confirmation.

7. If the signal in over 25% of the nucleus is too weak, the region should not be analyzed.

[Method Limitations]

This kit is only suitable for detection of AML1/ETO gene rearrangement in AML1 patients by fluorescence in situ hybridization, not for detection of other gene mutation modes.

[Cautions]

1. This kit is only used for in vitro diagnosis and must be used by strictly trained professionals.

2. In order to obtain ideal results, it is necessary to ensure that the reagents are correctly prepared and stored in accordance with the instructions.

[Symbols]

Symbol	Used for	Symbol	Used for
	Use-by date		Consult instructions for use
	Batch code		In vitro diagnostic medical device
	Temperature limit		Manufacturer
	Avoid overexposure to the sun		Date of manufacture

[Basic Information]

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