BCR/ABL Gene Fusion Kit（FISH）

Instruction For Use

[Product Name]

BCR/ABL Gene Fusion Kit (FISH)

[Specification]

5 Tests/box, 10 Tests/box, 20 Tests/box.

[Intended Use]

This product is used to qualitatively detect BCR/ABL gene fusion in patients with hematological tumors.It is used to assist diagnosis (BCR/ABL fusion gene can be found in more than 90% of CML and is a specific marker of CML) and targeted medication (BCR-ABL fusion gene can be used to guide the use of targeted therapy drugs Gleevec, treatment plan Selection and evaluation of drug efficacy). In addition, the dynamic changes of the BCR-ABL gene transcription level of patients can predict the recurrence of leukemia, which is a reliable indicator of the prognosis of leukemia.

[Principle of Detection]

Fluorescent In Situ Hybridization (FISH) enables a specific nucleotide fragments in cells clearly displayed by fluorescence. The FISH test process involves the melting of double-stranded DNA, and fluorescently labeled DNA probes can Combine with target sequence. After hybridization, unbound probes are washed away, the excess probes are washed away, and at the same time, the cell nucleus is stained with the counterstain 4',6-diamidino-2-phenylindole (DAPI) to emit blue fluorescence.

After the hybridization of the BCR/ABL fusion gene detection probe, two BCR green signals and two ABL orange signals can be observed in the natural state. Conversely, if the BCR/ABL region merges, the orange and green signals merge into yellow signals.

[Main Components]

Contains BCR/ABL probe(Contains Fluorescence-labeled probes, formamide, SSC, dextran sulfate) and DAPI counterstain solution(Contains DAPI, anti-fading liquid, glycerin).

[Storage and Validity]

BCR/ABL Fusion Probe and DAPI should be stored at -20°C in dark, sealed conditions, It should not be mixed with toxic, polluted and unpleasant odor items.

After opening, store at 2-8°C in a dark and sealed conditions; if it cannot be used up within 24 hours, please store it at -20°C ±5°C in a dark and sealed conditions;

The validity period is one year from the date of production.

[Applicable Instrument]

The probe hybridization of this kit needs to be hybridized on a hybridization instrument.

Objective lens: In FISH analysis, it is recommended to use 100 times achromatic objective lens of immersion type, which can obtain satisfactory results.

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

Optical Filters: It is recommended that customers use the probe to find out the details of the filter set used by the filter set supplier in order to select the filter suitable for labeled fluorescent stains.

Green fluorescence: excitation wavelength is 496nm , and emission wavelength was 520nm, Similar to FITC

Red fluorescence: excitation wavelength was 551nm ,and emission wavelength was 572nm, Similar to Rhodamine.

[Specimen Requirements]

Bone marrow or peripheral blood (2-3ml), heparin anticoagulant, the sample size depends on the specific situation of the patient, if the patient with aplastic anemia has fewer white blood cells, should be increased collection.

Treatment within 2 hours after collection, specimen transport and preservation is recommended at 2-8℃, no more than 72h.

[Test Method]

Sample collection

1. 2~3mL bone marrow or peripheral blood was collected(Heparin sodium anticoagulation), centrifuge at 2000rpm for 5 minutes, the supernatant was removed;
2. Take 1ml of cells and add 10ml of 0.075mol/L KCl to mix well by pipetting, and let stand for 2 minutes;
3. Constant temperature water bath 37±1℃ hypotonic for 20 minutes;
4. Add 1ml of fixing solution, mix by pipetting and pre-fix for 10 minutes at room temperature;
5. Mix by pipetting and centrifuge at 2000 rpm for 5 minutes.
6. the supernatant was removed, add 10ml fixative to the precipitate, mix by pipetting, and precipitate at -20℃ for 30 minutes;
7. Centrifuge at 2000rpm for 5 minutes, the supernatant was removed;
8. The above washing steps can be repeated until the cell pellet is washed out (repeated washing does not need to stand for 30 minutes).

slide preparation

1. Take a clean glass slide;

2. After resuspending the cells, take 3μl, drop the suspension onto the glass slide, and dry at room temperature;

3. Observe the cell density under a phase-contrast microscope with a 10× objective lens. It is required that the cells have no overlap, and the number of cells in a single field of view should be between 100 and 200;

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

3.2 If the cells overlap, add appropriate fresh fixative to dilute the cell suspension

3.3 If the cell density is low, centrifuge at 2000 rpm for 5 minutes, carefully aspirate the appropriate amount of supernatant, and after mixing, take another 3μl of suspension to prepare a slide, dry the slide, and observe

4. Observe under a phase-contrast microscope, if there is too much cell debris, you need to pre-treat and select the appropriate hybridization area.

Note: At least one extra slice should be prepared for each case, and the cell drops should be placed in an airtight container with absolute ethanol for a while, and it can be stored at -20℃±5 for 12 months. The remaining cell suspension can be stored at 2～8℃ for one month, and if necessary later, it can be remade.

Pretreatment of slides (the following two treatment methods are acceptable)

Method 1: Fast processing

1. Place the dropped glass slide in 2×SSC solution at room temperature and soak for 2 minutes;

2. Then soak in 70%, 90%, and 100% ethanol at room temperature for 2 minutes for dehydration;

3. Finally, take out the slide and let it dry at room temperature.

Method 2: Pepsin digestion treatment method

1. Pepsin solution configuration: Take 50ml of pepsin buffer and place it in a 37±1℃ water bath. 10 minutes before use, dissolve 0.1g of pepsin in the above-prepared pepsin solution and add all of it to 50ml pepsin solution and mix well, Replace after one day of use;

2. Place the slides in 37±1°C pepsin solution for digestion for 1 to 3 minutes (the enzyme efficacy can be determined through preliminary experiments);

3. Take out the slide, process the slides by 70%, 90% and 100% ethanol in sequence for 2 min, dry the slides at room temperature.

Note: When the sample is difficult to handle, such as excessive impurities, weak signal, etc., you can choose Method 2 for slide pretreatment.

Denaturation and hybridization of sample and probe (be careful to avoid light)

1. Remove the PML/RARA probe from the refrigerator, centrifuge instantly, and add 10μL to the target area with a pipette, Cover the specimen with coverslip to avoid bubbles.

2. Mount the slide with rubber cement along the edge of the cover glass. Pay attention to the complete mounting to prevent the hybridization solution from evaporating.

3. Place the slide on the hybridization machine and perform denaturation and hybridization according to the set procedure.

Wash after hybridization1(Keep away from light)

1. 30min before washing, put the prepared washing buffer I in a water bath and preheat to 72±1℃.

2. Take out the slide glass, remove the rubber glue, and incubate the slide glass in washing buffer II at room temperature for 5 minutes to remove the cover glass.

3. Put the slides in 72±1℃ Washing Buffer I for 2min;

4. Remove slide, and put slide into washing buffer II at room temperature for 2min.

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

6. DAPI staining and mounting: Add 10μL DAPI to the target area of the slide and cover with a coverslip, incubate at -20℃ in dack for 15 min.

7. Microscopic examination:Store the slices in a dark place. Store at -20℃ for long-term storage, Counting under a fluorescence microscope.

[Positive judgment value and Reference range]

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

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

3. Do not analyze areas with uneven hybridization.

4. Do not analyze if the outline of the nucleus is unclear or overlaps.

5. Do not analyze the area where the background depth 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.

8. If signals are present in more than 10% of the cytoplasm, the region should not be analyzed.

9. First determine the adequacy of the slide hybridization

Nuclear morphology: The boundary of the cell nucleus is usually clear, and the cell nucleus is in a complete form;

Background status: The background should not contain particulate matter that affects the count;

Fluorescence signal intensity: The signal should be bright, obvious and easy to calculate. The signal should exist in bright light, oval shape. Avoid excessively scattered signals.

10. Fluorescence signal observation of target area.

Use a 40× or 100× objective lens and a suitable filter to observe the BCR/ABL signal. Adjust the focus to observe the size and shape of the target signal and noise (non-target hybridization signal). Ensure that the background does not have a strong fluorescent interference signal.

Scan the entire slices to observe the overall fluorescence signal distribution of the cells, and select a more representative area for counting.

11. Select cells in the target area and count

Select an area with better cell nuclei distribution (such as the area that can distinguish a single cell nucleus), and ensure that the selected area can represent the observed signal distribution characteristics; start to analyze the cells and record the fluorescent signal characteristics of each cell; repeat the above operations until Count up to 200 cells;

12. Signal counting rules

12.1 Adjust the focus to find the signal in the nucleus. Analyze the selected cells with clear boundaries.

12.2 Cells are negative in the following cases (the BCR/ABL gene is not fused):

In normal cells, there are two orange signals and two green signals.

12.3 Cells are positive in the following cases (BCR/ABL gene fusion):

At least one set of orange and green fluorescent signals fused into a yellow signal.

[Method Limitations]

This kit uses fluorescence in situ hybridization to detect BCR/ABL fusion gene detection in patients with hematological tumors, and cannot be used to detect other gene mutation methods.

[Cautions]

1. This kit is an in vitro diagnostic reagent and can be reused.
2. During the experimental operation of this kit, it is necessary to wear latex gloves to avoid contact with the skin. In case of accidental contact, rinse immediately with plenty of water.
3. The discarded samples and experimental wastes during the experiment shall be recycled and treated as medical waste.
4. 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

	The date by which the device should be used		Any special operating instructions
LOT	Batch code	IVD	In vitro diagnostic medical device
	Temperature limit		Name and address of manufacturer
CE	CE mark	EC REP	Authorized representative in the European Community
	Keep away from sunlight	REF	Reference number
NON STERILE	Non-sterile		

[Basic Information]

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