IGH Gene Breakpart Kit（FISH）
Instruction For Use

[Product Name]

IGH Gene Breakpart Kit（FISH）

[Specification]

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

[Intended Use]

It was used to qualitatively test the gene fusion of IGH gene for hemooncology patients. The kit is only for research to use.

Breakpart and translocation of the immunoglobulin heavy chain (IGH) gene (14q32) are common in ALL/MM/lymphoma, occur in 50% of B-cell NHL and in a variety of other lymphoma types. The types of translocation are complex. The mutual translocation of IGH and specific genes is helpful for clinical differential diagnosis and prognosis judgment. For example, the MYC/IGH fusion gene can be used to assist in the diagnosis of Burkitt lymphoma (BL) (75% incidence), and guide the treatment of high-grade B-cell lymphoma. The BCL1/IGH fusion gene occurs in 75% of mantle cell lymphomas and can be used to assist in the diagnosis of this tumor. The BCL2/IGH fusion gene occurs in 85% of follicular lymphomas (FL) and 1/3 of diffuse lymphomas (DL).

The kit is only for research to use.

[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.

In the natural state, IGH gene region can be observed as a fused red and green signal. Conversely, if there is a breakpart in the 14q32 IGH gene region, the red and green signals are separated.

[Main Components]

The kit is mainly composed of IGH 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:

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 -20℃ glass 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℃±5. The remaining cell suspension can be stored at 2-8℃ for one month, and can be prepared again if necessary.

Slide Pretreatment:

1. Method 1: Rapid Processing

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

1.2 Dehydration: the slides were successively treated in 70%, 85% and anhydrous ethanol solution for 2 min.

1.3 Remove the slide and then dried at room temperature.

2. Method 2: pepsin digestion

2.1 pepsin solution preparation: 500 μl of 1M HCl, add it into 50ml purified water, placed in a 37±1℃ water bath. 10 minutes before use, add dissolved 0.1g pepsin to the 50ml prepared pepsin solution, mix well. Replace after one day of use.

2.2 Soak in the 1×PBS solution at 37±1℃ for 5min.

2.3 Remove the slide, and then put it into 37±1℃ pepsin solution to digest for 1-5min (enzyme efficacy can be determined by preliminary experiment)

2.4 Remove the slide, wash in the 1×PBS solution for 2-3min at room temperature

2.5 Remove the slide, fix them in 1% paraformaldehyde/PBS for 10 minutes at room temperature

2.6 Remove the slide, wash in the 1×PBS solution for 2-3min at room temperature

2.7 Remove the slide, the slides were successively treated in 70%, 85%, and anhydrous ethanol solution for 2 min. Remove the slide, dried at room temperature.

Note: When the sample is difficult to process such as too many impurities, weak signal, etc., method 2 can be used for slide pretreatment.

Denaturation and hybridization (avoid light):

1. Remove the probe,mix and centrifuge. Take 10ul 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 to denature and hybridize.

Washing after hybridization:

1. 30 minutes before washing. Put the washing buffer I into the water bath and heat it to 72±1℃.

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 5 minutes, and remove the coverslip.

3. Put the slide into washing buffer I at 72±1℃, placed for 2 minutes

4. Remove the slide, soak in the washing buffer II solution at room temperature, placed for 1min

5. Remove the slide in 70% and 85% ethanol solution for 2min in turn

6. Add 10ul DAPI to the target area of the slide and cover with a coverslip. The slides were placed in -20℃ condition for 10-20 minutes and then taken out for observation and analysis under a fluorescence microscope.

[Results Positive judgment and reference interval]

It is recommended to count 200 cells (excluding overlapping cells) and count the IGH gene breakpart.

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

Anormal cells: The type of intracellular signal is reflected in the separation of red signal and green signal, which means breakpart.

[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.

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

9. First judge the sufficiency of slide hybridization

Nuclear morphometry: The borders of the nucleus are usually clear and the nucleus is intact

Background state: The background should not contain particulate matter that affects the counts.

Fluorescence Signal Intensity: The signal should be bright, distinct and easy to count. The signal should exist in a bright, oval shape. Avoid overly scattered signals.

10. Fluorescence signal observation of the target area

Observe IGH signal with a 40× or 100× objective lens and appropriate filters. Adjust the size and shape of the focus to observe the target signal and noise (non-target hybridization signal). Make sure that the background does not have strong fluorescence interference signals.

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

11. Select and count cells within the target area

Select an area with better distribution of nuclei (such as areas that can distinguish single nuclei), and ensure that the selected area represents the observed signal distribution characteristics; Start analyzing and recording the fluorescence signal of each cell. Repeat the above operation until 100 cells are counted.

12. Signal counting rules

12.1 Adjust the depth of focus to locate the signal in the nucleus. Analyze the selected cells with sharp boundaries.

12.2 The following cells are negative (IGH gene non-rearrangement):

There are two fusion signals in the nucleus, that is, the red and green signals are linked together.

12.3 The following cells are positive (IGH gene rearrangement):

At least one set of red and green fluorescent signals are separated.

[Method Limitations]

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

[Product Performance]

1. Localization of metaphase chromosome probe

The IGH orange and IGH green fluorescent probes, the separation probe of IGH dual color were shown to bind to the 14q32 chromosomal extension, in all eight metaphases, and would not hybridize to other positions.

2. Sensitivity and specificity

Sensitivity was defined as the percentage of chromosomal targets with expected normal signal patterns. Specificity was defined as the percentage of signal bound to the correct location.

The specificity and sensitivity of the IGH orange and IGH green fluorescent probes were evaluated by interphase chromosomes. There are 6 specimens (6 slides) from peripheral blood cell cultures of 5 normal donors.

For sensitivity assessment, the signals from the IGH orange and IGH green fluorescent probes, count chromosomes per interphase division (normal: 2 signals). It is counted 20 metaphase cells per slide. The expected signal value for each probe is 240 (see Table 1). For specificity assessment, the number of metaphases with expected fluorescent signal was 120 in total (see Table 2).

Table 1 Sensitivity Analysis

Probe	Metaphase chromosome fluorescence signal number		Sensitivity
	Observed value	Expected value	point estimate percentage
IGH Red	240	240	100
IGH Green	240	240	100

Table 2 Specificity Analysis

Probe	Metaphase with expected fluorescent signal			Specificity
	Non-specific hybrid metaphase cells	Specific hybrid metaphase cells	Expected value	point estimate percentage
IGH Red	0	120	120	100
IGH Green	0	120	120	100

[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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