BCL2(18q21) Gene Breakpart Kit（FISH）

Instruction For Use

[Product Name]

BCL2(18q21) Gene Breakpart Kit (FISH)

[Specification]

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

[Intended Use]

This reagent is used for in situ hybridization staining based on routine staining to provide auxiliary information for physicians in diagnosis.

[Principle of Detection]

Fluorescent In Situ Hybridization (FISH) enables a specific nucleotide sequence in a cell to appear clearly. The fluorescence-labeled DNA probes can bind to the target sequence to emit fluorescence, and the DNA fragments after hybridization can be directly observed under a fluorescence microscope. After 10% neutral buffered formalin-fixed and paraffin-embedded tissue is placed on the glass slide, the DNA is first denatured into single-stranded DNA, and then it is combined with the probe DNA in an orderly manner. After the binding is completed, the excess probe DNA is washed away by the washing buffer, and at the same time, the cell nucleus is stained with the specific DNA staining agent 4',6-diamidino-2-phenylindole (DAPI) to emit blue fluorescence. The probe signal after hybridization can be observed under a fluorescence microscope.

[Main Components]

Contains BCL2 Fracture Probe(Contains Fluorescence-labeled probe, formamide, sodium chloride, trisodium citrate, dextran sulfate), and DAPI counterstain solution(Contains DAPI, p-phenylenediamine, glycerin).

Components not included but necessary for the test:

Fluorescence in situ hybridization sample processing kit(Contains pretreatment buffer, pepsin solution, washing buffer I, was hing buffer II).

Other reagents not provided:

Xylene; Ethanol (anhydrous, 85%, 70%); Deionized water; Rubber glue.

[Storage and Validity]

stored at -20℃±5℃ away from light,valid for 12 months.

Transportation conditions: Dry ice is added during transportation, and the transportation temperature does not exceed 8°C.

[Applicable Instrument]

Fluorescence in situ hybridization system;

Fluorescence microscope, in which:

Objective lens: 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.

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

[Specimen Requirements]

Sample tissue of lymphoma fixed with 10% neutral buffer formalin for 6 ~ 48h. According to the requirements of pathological technical specifications, sampling, dehydration, paraffin embedding into paraffin block. Paraffin blocks should be stored in a special, ventilated and dry paraffin block cabinet.

The tissue sections with a thickness of 3 ~ 5 μm were spread on sticky slides. Remove the excess water in the tissue sections, the sections were then placed in a drying oven at 60℃ (±5℃) for 30 ~ 60min or placed overnight at 37℃, then remove it from the thermostat and let it cool down at room temperature.

If the tissue slices are stored at room temperature, the detection should be completed within 7 days;If the tissue sections in cold storage (2 ~ 8℃), the detection should be completed within 1 months

[Test Method]

Device and equipment required for testing

Fluorescence in situ hybridization system; Fluorescence microscope; Pressure cooker; Centrifugal; Water bath; Induction cooker.

Detection method

1. Sample processing and slide preparation

1.1 The sample is fixed in 10% neutral buffered formalin buffer at room temperature for 6 to 48 hours. In order to achieve the best and uniform fixation and paraffin embedding effect, the sample size should not exceed 0.5cm^3.

1.2. Standard operation and paraffin embedding, using high quality paraffin. Infiltration and embedding should be performed below 65℃.

1.3. Cut into slices with a thickness of 3 to 5 μm.

2. slice preprocessing program

2.1. Baking slices at 55℃～65℃ for more than 3h (overnight).

2.2. Preheat the pepsin solution in advance to bring the temperature to 37°C and keep it for use.

2.3. Soak 3 times in xylene at room temperature, 10min/time.

2.4. Soak 2 times in absolute ethanol at room temperature, 5min/time.

2.5. The sections were processed in 85% and 70% ethanol at room temperature for 3 minutes.

2.6. Clean with deionized water 3 times, 2min/time.

2.7. Use an induction cooker to boil an appropriate amount of pretreatment buffer, and then keep it in a heat preservation state, put the slices in the repair for 20 minutes, or add an appropriate amount of pretreatment buffer to the pressure cooker, and repair at high pressure for 5 minutes.

2.8. Take out the section and wash 3 times with deionized water. Put the slices directly into the pepsin solution and digest for 15±10min.

Note: The digestion time varies according to different tissue (cell) types and slice thicknesses. Generally speaking, it is recommended to pre-experiment to find the best digestion time for the sample.

2.9. Clean with deionized water 3 times, 2min/time.

2.10. Dehydration

Treat them in 70% ethanol, 85% ethanol, and anhydrous ethanol solution for 2 minutes, and then air dry.

3. FISH operation steps

Note: The fluorophore will cause photobleaching when exposed to strong light. Protecting reagents and slices containing fluorescent groups from light can help reduce this effect. All steps that need to be protected from light are carried out in a dark place. Please take precautions during the operation of the FISH experiment. The reagents should not be in direct contact with the skin.

3.1. Sample denaturation and hybridization

3.1.1 The hybridization solution containing the BCL2 fragmentation probe was taken out of the refrigerator, centrifuged momentarily, and 10 μL of each sample was taken with a pipette. Drop into the target area.

3.1.2 Cover the sample with a cover glass to avoid air bubbles and seal it with rubber glue.

3.1.3 Put the slices into the hybridization machine and denature at 83℃±2℃ for 6min, hybridize overnight at 40℃.

Note: The slices cannot be dried during hybridization!

3.2 Treatment after hybridization

3.2.1 Need to heat the washing buffer I in a water bath 30min in advance to 73℃±1℃, remove the rubber glue carefully.

3.2.2 Place the slices in washing buffer II for 5-10 minutes, and remove the cover glass.

3.2.3 Place the slices in washing buffer I (heated in a water bath to 73 ℃ ± 1 ℃), lift the slices up and down for 1 to 3 seconds, and leave them for 3 minutes.

3.2.4 At room temperature, place the slices in washing buffer II, pull up and down for 1 to 3 seconds, and process the slices for 2 minutes.

3.2.5 Place them in 70% and 85% ethanol solution for 2 minutes.

3.2.6 Dry the sample in the dark.

Note: It is best to place 4 slices in the Coplin jar at the same time each time, the timing starts when the last slice is placed in the Coplin jar.

4. DAPI staining and mounting

Add 10μL DAPI to the paraffin sections to avoid air bubbles, cover with coverslip, and incubate at -20°C in the dark for 10-20 min.

5. Microscope examination

The slides were taken out for observation and analysis under a fluorescence microscope (for long-term storage, they should be stored at -20°C).

【Positive judgment value】

Count 100 tumor cells and analyze the BCL2 gene breakage in the sample. When the number of abnormal cells is more than 15%, it is judged as positive, the BCL2 gene is broken. When the number of abnormal cells is ≤15%, it is judged as negative, and the BCL2 gene is not broken.

[Interpretation of results]

In normal cells, there are two fused orange and green signals, indicating that the BCL2 gene is not broken; in abnormal cells, there are at least one set of orange and green separation signals, indicating that the BCL2 gene is broken.

Precautions for result analysis:

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.

[Method Limitations]

1.This kit is an in vitro diagnostic reagent. It is suitable for the detection of BCL2 gene abnormalities in patients, and cannot be used for the detection of other gene mutation methods.

2.The detection results of this kit are for clinical reference only, and the clinical judgment of the detection results should be comprehensively evaluated in conjunction with the patient's medical

history and other clinical diagnosis results, and cannot be used as the only basis for clinical diagnosis and treatment.

3. If the test results are not consistent with the cytopathological characteristics, the pathological diagnosis should be verified or retested.

4. The performance index of this product is obtained based on the test procedure described in the manual. Changes to this procedure may change the result of the test.

[Product Performance]

1. Fluorescence signal intensity

After the probe is hybridized with a control section made of lymphocytes cultured in peripheral blood, it should emit a fluorescent signal that can be recognized by the naked eye under a fluorescent microscope.

2. Sensitivity

On control sections made of lymphocytes cultured in peripheral blood, 100 chromosomes 18 of 50 metaphase cells were analyzed, and at least 98 of them showed an orange-green fusion signal marked by a BCL2 gene locus.

3. Specificity

On control sections made of lymphocytes cultured in peripheral blood, 100 chromosomes 18 of 50 metaphase cells were analyzed, on the long arm of chromosome 18 (18q21), and at least 98 of them showed an orange-green fusion signal marked by a BCL2 gene locus.

[Cautions]

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

2. This reagent can be reused.

3. 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.

4. The discarded samples and experimental wastes during the experiment shall be recycled and treated as medical waste.

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

6. Please read this manual carefully before use.

[Symbols]

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

[Basic Information]

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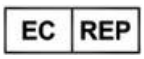

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