C-MYC Gene Breakpart Kit（FISH）

Instruction For Use

[Product Name]

C-MYC Gene Breakpart Kit (FISH)

[Specification]

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

[Intended Use]

This kit is used to qualitatively detect the diagnosis of C-MYC gene rearrangement in lymphoma. To guide the treatment and prognosis evaluation of lymphoma, not for disease screening or diagnosis.

[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.

When the hybridized C-MYC gene recombination probe is generated, it can be observed that the C-MYC gene region will become a fused orange and green signal in a natural state. Conversely, if the C-MYC area is broken: the orange and green signals separate.

[Main Components]

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

[Storage and Validity]

C-MYC gene break 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, such as ThermoBrite.

The probe of this kit needs to be observed and analyzed under a fluorescence microscope. The configuration of the required fluorescence microscope includes:

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.

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

[Specimen Requirements]

Formalin-fixed, paraffin-embedded lymphoma tissues and cells. Samples should be collected according to laboratory standard procedures, and the selection of test kit samples should be performed by pathologists. The sample should avoid contact with acids and strong bases, and avoid overheating.

The selected test sample should generally be subjected to FISH experiment within six weeks after preparation, otherwise it may affect the effect of the experiment.

[Test Method]

Sample processing and slide preparation

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.

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

3.Cut into slices with a thickness of 3 μm.

Slice preprocessing program

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

2. Preheat the pepsin solution(2mg/ml, 10mM HCl) in advance to bring the temperature to 37°C and keep it for use.

3. Place the slices in xylene at room temperature for 10 minutes, and repeat this step three times.

4. Place the slices in absolute ethanol at room temperature for 5 minutes, and repeat this step once.

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

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

7. Use a microwave oven 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.

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: According to different factors, such as slice fixation conditions and process, slice thickness, tissue/cell characteristics, and digestion time, we recommend: digestion for tissue section samples for 5-25 minutes, for cell sample digestion 3- 8 minutes. Generally speaking, it is recommended to pre-experiment to find the best digestion time for the sample. And each digestion does not exceed 4 slices.

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

10. Dehydration: treat them in 70% ethanol, 85% ethanol, and anhydrous ethanol solution for 2 minutes, and then air dry.

FISH operation steps

Warning and prevention: 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.

(1)Sample denaturation and hybridization

1. The hybridization solution containing the C-MYC fragmentation probe was taken out of the refrigerator, centrifuged momentarily, and 10 μl of the solution was pipetted to the target area. Drop the probe evenly to the entire target area.

2. Cover the sample with a cover glass(22 mm× 22 mm) to avoid air bubbles and seal it with Mounting glue.

3. Put the slices into the hybridization machine and denature at 80℃±2℃ for 6min, hybridize overnight at 40℃(It is recommended to denature at 82℃ for 6 minutes and hybridize at 40℃ overnight).

Note: The slices cannot be dried during hybridization!

(2)Treatment after hybridization

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

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

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 2 minutes.

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 3 minutes.

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

6. Dry the sample in the dark.

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

(3) Observed

1. Add 10μL DAPI to the paraffin sections to avoid air bubbles, cover with coverslip, and incubate at -20°C in the dark for 15 min.

2. Observe and analyze under a fluorescence microscope.

Analysis of FISH test results

1. Normal cells: Two yellow signal points formed by the fusion of orange and green signals in a single interphase cell nucleus.

2. Abnormal cells: There is more than one orange and green signal separated, which is abnormal breakage.

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. When analyzing paraffin sections, the area to be analyzed should be where the tumor cells are concentrated (to be identified by the pathologist).

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

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

[Method Limitations]

This kit uses fluorescence in situ hybridization technology for C-MYC gene rearrangement detection,and cannot be used for the detection of 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
	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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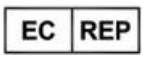

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