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Product Cat. No.: FP-007 **For Clinical Diagnosis & Scientific Research.**

Kappa chain probe detection kit
Instructions Manual

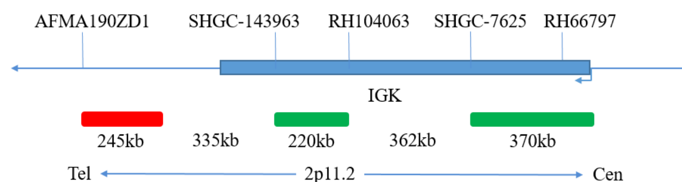
[Product Name] Kappa chain probe detection kit.
[Package Specifications] 10 Tests/box.
[Intended use]
This kit uses fluorescence in situ hybridization to detect kappa gene status in vitro. The detection samples are B-cell lymphoma surgical resection or biopsy tissue paraffin embedded samples. This kit is only applicable to the detection of kappa gene rearrangement status and provides doctors with auxiliary information for diagnosis.
The most common genetic abnormality in B-cell lymphoma is the heterotopic and rearrangement of immunoglobulin heavy chain (IGH) sites. In addition, 5-10% of B-cell lymphomas have heterotopic and rearrangement of immunoglobulin light chain. Immunoglobulin light chains include kappa chain (IGK) and lambda chain (IGL), and their positions on human chromosomes are 2p12 and 22q11, respectively. Current studies show that IGK rearrangement is acquired gene damage, and B-cell lymphoma cells are formed by the monoclonal proliferation of B cells with genetic abnormalities, so there are monoclonal changes. This monoclonal IGK gene rearrangement can be used as a specific molecular marker for the detection of B-cell lymphoma for the diagnosis of B-cell lymphoma, and this clonal detection is helpful to distinguish polyclonal reactive hyperplasia from malignant proliferative disease.
Therefore, the detection of IGK gene status has guiding significance for the treatment and prognosis of B-cell lymphoma.

[Detection principle]
This kit is based on fluorescence in situ hybridization technology. One nucleotide of the nucleic acid probe is labeled with fluorescein. The detected target gene and the nucleic acid probe are homologous and complementary. After denaturation, annealing and renaturation, the hybrid of the target gene and the nucleic acid probe can be formed. Through the fluorescence detection system, qualitative, quantitative or relative positioning analysis of the target gene under the microscope.
This kit uses rhodamine fluorescein (rho) - labeled orange probe and fluorescein isothiocyanate (FITC) - labeled green probe. The two probes can be combined to the target detection site by in situ hybridization. Under normal conditions (no rearrangement of kappa gene), these two probes hybridize and bind to the same gene, and are displayed as green and orange signals close to each other or yellow signals overlapping each other under fluorescence microscope. When there is a gene rearrangement, the green and red signals are "broken" due to the replacement of the recombination fusion partners, and appear as monochromatic signals separated far away. The rearrangement of kappa gene in the tissues of B-cell lymphoma patients was detected in vitro by this method of fracture fluorescence in situ hybridization, which provides a reference basis for the treatment, prognosis and medication of B-cell lymphoma patients.

[Product Composition]
This kit is composed of kappa break apart probe hybridization solution as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
kappa break apart probe	100μL/Tube	1	IGK Orange probe IGK Green probe



[Storage conditions & Validity]

Keep sealed away from light at -20ºC±5ºC. The product is valid for 20 months. Within 24 hours for short-term preservation, keep sealed at 2-8ºC in dark. For long-term preservation after opening, keep the lid sealed at -20ºC±5ºC away from light. The kit is transported below 0ºC.

[Applicable Instruments]

Fluorescence microscopy imaging system including fluorescence microscopy and filter sets suitable for DAPI, Green, and Orange.

[Sample Requirements]

1. Applicable specimen type: B-cell lymphoma paraffin embedded tissue sample.
2. The specimen shall be fixed with 4% neutral formalin fixed solution within 1 hour after being isolated. After the specimen is fixed, it shall be regularly dehydrated and embedded in paraffin.
3. The thickness of the paraffin section will affect the experimental results, and the section thickness is 4~5μm is appropriate.
4. For paraffin embedded tissue samples, representative tumor tissue wax blocks shall be selected and confirmed by HE staining.
5. It is recommended to select paraffin embedded tissue specimens (within 5 years) with shorter storage time.

[Test method]

1. Related Reagents

The following reagents are required for the experiment but not provided in this kit

① 20×SSC, pH 5.3±0.2

Weigh 176g of sodium chloride and 88g of sodium citrate, dissolve in 800mL of deionized water, adjust the pH to 5.3±0.2 at room temperature, and complete to 1 L with deionized water. High-pressure steam sterilization, stored at 2-8ºC, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

② 2×SSC, pH 7.0±0.2

Take 100mL of the above 20xSSC, dilute with 800mL deionized water, mix, adjust the pH to 7.0±0.2 at room temperature, complete to 1L with deionized water, stored at 2-8ºC, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

③ Ethanol Solution: 70% ethanol, 85% ethanol

Dilute 700ml, 850ml of ethanol with deionized water to 1L. The shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

④ Pepsin

Gastric storage solution: weigh 5g pepsin dry powder and dissolve it in 100ml distilled water, shake it gently until it is completely dissolved, and store it at - 20 ℃, with a shelf life of 6 months.

Pepsin working solution (0.5%): dissolve 5ml pepsin storage solution in 45ml HCl solution (pH = 2), mix well, and prepare it for use..

⑤ 0.3% NP-40/0.4xSSC solution, pH 7.0-7.5

Take 0.6mL NP-40 and 4mL 20×SSC, add 150mL deionized water, mix, adjust the pH to 7.0-7.5 at room temperature, with deionized water complete to a volume of 200mL. Stored at 2-8ºC, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑥ Diamidinyl phenylindole (DAPI) counterstain

Use commercially available anti-quenching DAPI counterstain.

2. Sample collection and slides preparation

It is suggested to select known positive and negative specimens as external control.

① Section preparation: tissue sections fixed in neutral formalin and embedded in paraffin are placed on clean sections.

② Baking slides: place tissue slices on the baking machine at 65℃ overnight to bake slices, and aging slices.

③ Dewaxing: immerse the tissue sections in the dye vat containing xylene for 10 min, repeat it once, and then immediately immerse them in 100% ethanol for 5 min.

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④ Rehydration: at room temperature, the tissue sections were placed in 100% ethanol, 85% ethanol and 70% ethanol for 2min respectively, and then immersed in deionized water for 3min. After taking out the sections, the excess water was absorbed along the tissue with a lint free paper towel.
⑤ Water treatment: immerse tissue sections in deionized water for 30~40min under 95℃ water bath (deionized water shall be preheated by water bath).
⑥ Washing: at room temperature, tissue sections were immersed in 2 × SSC solution, rinse twice for 5min each time.
⑦ Protease treatment: immerse the tissue sections in the preheated 37℃ protease working solution for 15~40min (the protease working solution is ready for use and discarded after one use).
⑧ Washing: at room temperature, tissue sections were immersed in 2 × SSC solution, rinse twice for 5min each time.
⑨ Dehydration: the tissue sections were placed in 70% ethanol, 85% ethanol and 100% ethanol for 2min respectively, and then dried naturally.

3. Denaturation and Hybridization

The following operations should be performed in a darkroom.
① Take out the probe put at room temperature for 5min. Mix and centrifuge briefly. Take 10μl droplet in the cell and drop in the hybridization zone, immediately cover 22mmx22mm glass slide area; spread evenly without bubbles the probe under the glass slide covered area and seal edges with rubber (edge sealing must be thorough to prevent dry film from affecting the test results during hybridization).
② Place the glass slides in the hybridization instrument, denature at 85°C for 5 minutes (the hybridizer should be preheated to 85°C) and hybridize at 42°C for 2 to 16 hours.

4. Washing

The following operations should be performed in a darkroom.
① Take out the hybridized glass slides, remove the rubber on the coverslip and immediately immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.
② Place the slides in a 2×SSC at room temperature for 1 min.
③ Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2) solution and wash for 2min.
④ Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

5. Counterstaining

The following operations should be performed in a darkroom
10μl of DAPI counterstaining agent was dropped on the hybridization area, and the sections were covered immediately. After being placed in the dark for 10-20min, the sections were observed with a suitable filter under a fluorescence microscope.

6. FISH results observation

The counterstained sections were placed under a fluorescence microscope, and under natural light, they were first stained with a low-power objective lens (10x), B-cell lymphoma cell region was confirmed below; Go to 40×, under the objective lens, find a position where the cells are evenly distributed; In the high-power objective lens (60x, 100x). The cells with uniform nuclear size, complete nuclear boundary, uniform DAPI staining, no overlapping nuclei and clear signals shall be selected. At least 100 tumor cells shall be randomly selected to count the red and green signals in the nuclei.

[Positive judgment value or reference interval]

1. Signal classification and counting

① Normal cell signal: there are 2 fused yellow signals in a single interphase nucleus.
② Abnormal cell signal: there is one orange red signal, one green signal and one yellow signal in the nucleus of a single interphase.
Note: since the green probe marks two sections, the green signal may be displayed as a pair of signal points.

100 cells are randomly counted and the number of normal signal cells and abnormal signal cells are counted. Each cell is counted once. Only cells with hybridization signals (both color signals) are counted. Cells with no signal or only a single color signal are not counted. Cells with weak signal or too diffuse signal are not counted.

2. FISH result judgment

To determine the abnormality of the detection result, it is necessary to establish an abnormality threshold

① ***Establishment of abnormal threshold***

1) It is suggested to select 20 tissue samples from patients with non-B-cell lymphoma as negative control.

2) Slides were prepared using the above methods and steps for FISH experiments.

3) Establishment of abnormal threshold: analyze 100 cells per sample, count the percentage of abnormal signals in each probe group, and calculate the average value and standard deviation of the percentage of cells showing abnormal signals. The abnormal threshold is defined as the average value + 3 × Standard deviation.

Abnormal threshold = average (m) + 3 × Standard deviation (SD)

Example: Table 2: tissue samples from 20 patients with non-B-cell lymphoma were selected as negative controls for FISH detection.

Table 2: Establishment of abnormal threshold

No.	Abnormal cell (%)
Sample 1	5
Sample 2	3
------------	……….
Sample 20	4
average	3
SD	0.3
threshold	(Abnormal threshold = average (m) + 3 × SD)3.9

② ***Result judgment:***

If the detected value of the number of cells displaying the abnormal signal mode is greater than the abnormal threshold, it is determined as a positive result; If the detected value of the number of cells displaying the abnormal signal mode is less than the abnormal threshold, it is determined as a negative result; If the detection value of the number of cells in the abnormal signal mode is equal to the abnormal threshold, increase the number of cells in the observation sample and count 200 cells to judge the final result.

[Limitations of inspection methods]

This kit is used for B-cell lymphoma surgical resection or biopsy tissue paraffin embedded specimens, and is not recommended for other tissues. The detection ability of paraffin tissue samples that have been stored for too long cannot be evaluated according to this instruction; It shall be operated according to the procedures provided in this manual. Changing the procedures may change the inspection results; This kit only detects kappa gene status and cannot be used as the only basis for treatment, prognosis judgment or other clinical management of B-cell lymphoma patients. It needs to be comprehensively evaluated on the basis of medical history and other diagnostic results.

[Product performance index]

1. Fluorescence signal intensity: after the probe is effectively hybridized with the karyotype sample, the fluorescence signal that can be recognized by the naked eye shall be emitted under the fluorescence microscope.

2. Sensitivity: 100 chromosomes 2 of 50 cells in metaphase were analyzed, and at least 98 chromosomes 2 showed one orange red fluorescence signal and one green fluorescence signal.

3. Specificity: 100 chromosomes 2 of 50 cells in metaphase were analyzed, and at least 98 chromosomes 2 showed a specific orange red fluorescence signal and a specific green fluorescence signal in the target region.

[Precautions]

1. Please read this manual carefully before testing. The testing personnel shall receive professional technical training. The signal counting personnel must be able to observe and distinguish orange red and green signals.
2. When testing clinical samples, if it is difficult to count the hybridization signals and the samples are not enough to repeat the retest, the test will not provide any test results. If the amount of cells is insufficient for analysis, again, the test will not provide test results.
3. The formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity, so they need to be operated in the fume hood and wear masks and gloves to avoid direct contact.
4. The results of this kit will be affected by various factors of the sample itself, and also limited by enzyme digestion time, hybridization temperature and time, operating environment and limitations of current molecular biology technology, which may lead to wrong kappa rearrangement gene results. The user must understand the potential errors and accuracy limitations that may exist in the detection process.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical wastes and should be properly disposed of.

[References]

[1]. José I. Martín-Subero ‡, Lana Harder ‡, Gesk S, et al. Interphase FISH assays for the detection of translocations with breakpoints in immunoglobulin light chain loci[J]. International Journal of Cancer, 2002, 98(3):470–474.
[2]. Poulsen T S, Silahtaroglu A N, Gisselø C G, et al. Detection of illegitimate rearrangements within the immunoglobulin light chain loci in B cell malignancies using end sequenced probes[J]. Leukemia, 2002, 16(10):2148.
[3]. Kornblau S M, Goodacre A, Cabanillas F. Chromosomal abnormalities in adult non-endemic Burkitt's lymphoma and leukemia: 22 new reports and a review of 148 cases from the literature[J]. Hematological Oncology, 1991, 9(2):63-78.

[Basic information]

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[Manual Approval date & Revision date]

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