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Product Cat. No.: GBS-012

For Research Use Only.

Lambda chain probe detection kit Instructions Manual

[Product Name] Lambda chain probe detection kit.
[Package Specifications] 10 Tests/box.

[Intended use]

This product is mainly used for lambda chain detection of B-cell chronic lymphoproliferative disease (B-CLPD). The applicable population is the patients suspected or diagnosed with chronic lymphoproliferative disease of B cells by routine clinical examination.

B cell chronic lymphoproliferative disease (B-CLPD) is a group of mature B cell clonal proliferative diseases involving peripheral blood /bone marrow. Because most of the cells are small and medium-sized mature lymphocytes, it is often diagnosed or misdiagnosed as chronic lymphocytic leukemia (CLL) according to the morphological characteristics of the cells.

The confirmation of monoclonal is very important for the diagnosis of B-CLPD. The common methods of clonality detection are: ① flow cytometry (FCM): the clonality of B cells is mainly determined by detecting the restricted expression of SIG light chain in cells. Immunophenotypic features of malignant mature B cells: restricted expression of SIG light chain and abnormal expression of antigen. When κ (kappa)/ λ (lambda) > 3:1 or <0.3:1 suggests monoclonal. A few B-CLPD patients do not express κ and λ (CD19 positive and SIG negative cells > 25%) also suggested the monoclonal nature of B cells, and IGH or IGL (lambda) gene rearrangement should be tested if necessary. ② Genetics: routine karyotype examination and fluorescence in situ hybridization (FISH) were used to analyze clonal chromosomal abnormalities. ③ Molecular biology: detection of IGH 、 IGκ and Ig λ Gene rearrangement by PCR can judge the clonality abnormality of B cells. Therefore, Ig λ (IGL, lambda) gene rearrangement detection is of great significance for the diagnosis and differential diagnosis of B-CLPD.

This kit has not been clinically validated in combination with gene targeted therapeutic drugs, but only for gene detection performance. This kit is only applicable to the detection of B-CLPD and provides doctors with auxiliary information for diagnosis.

[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 orange red fluorescein to label the orange red probe and green fluorescein to label the green probe. The two probes can be combined with the target detection site by in situ hybridization technology.

Under normal conditions (no gene rearrangement occurred), two fused yellow signals were displayed under the fluorescence microscope. When there is a gene rearrangement, the fluorescence microscope shows one orange red signal, one green signal and one yellow signal. The gene rearrangement was detected by this method, which can provide reference for the clinical identification of B-CLPD patients, prognosis judgment and medication.

[Product Composition]

This kit is composed of IGL (lambda) dual color probe as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
IGL (lambda) dual color probe	100μL/Tube	1	IGL Orange probe IGL Green probe

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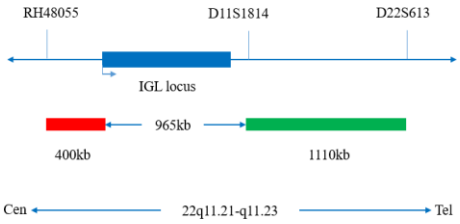

[Storage conditions & Validity]

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

[Applicable Instruments]

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

[Sample Requirements]

1. Applicable specimen type: fresh bone marrow specimen shall be stored at 4°C for no more than 24 hours and used for FISH detection after culture.
2. If the storage temperature of the sample is too high or too low (such as freezing), the sample will not be used for culture and should be discarded.
3. The bone marrow cell suspension for karyotype analysis should be stored at -20°C for FISH detection.
4. If the cell suspension is excessively volatilized or contaminated during storage, the sample shall be discarded.

[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 2-8°C, 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 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

③ 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 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

④ Fixation solution (methanol: glacial acetic acid = 3:1)

Prepare a ready to use fixation solution by mixing thoroughly 30ml of methanol and 10ml of glacial acetic acid.

⑤ PBS buffer, pH 7.4 ± 0.2

Sodium chloride	8g
Potassium chloride	0.2g
Sodium hydrogen phosphate	3.58g
Potassium dihydrogen phosphate	0.27g

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Dissolve the above reagents with 800ml deionized water, adjust the pH value to 7.4±0.2 at room temperature, and fix the volume to 1L with deionized water. It is stored at room temperature with a shelf life of 6 months. If the reagent is turbid or contaminated, it cannot be used.

⑥ 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.

⑦ 0.075M KCl solution

Weigh 2.8g of potassium chloride, dissolve in 400mL of deionized water and complete to 500mL with deionized water. Stored at room temperature, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑧ HCl solution

Measure 8.2ml of concentrated HCl, add deionized water to mix and constant volume to 100ml, and store at room temperature to obtain 1m HCl. Dilute to 0.01M according to 10 times dilution method as required.

⑨ 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 Sample collection: take heparin anticoagulated bone marrow.

② Harvest cells: aspirate uncultured bone marrow cells or cultured bone marrow cell samples to the tip bottom centrifuge tube, centrifuge, 1000 rpm, 10min, and remove the supernatant

③ Hypotonia: wash the cells once with PBS, centrifuge for 1000 rpm for 10min, remove the supernatant, add 6-8ml of 0.075mol/l KCl solution preheated to 37℃, blow and mix with a pipette, and then place it in a 37℃ incubator for 20-30min.

④ Pre fixation: add 2ml of 3:1 methanol and glacial acetic acid fixed solution, blow and mix, and centrifuge for 10min at 1000 rpm.

⑤ Fixation: aspirate the supernatant, add 5ml of freshly prepared 3:1 methanol and glacial acetic acid fixed solution, blow and mix, fix for 10min, and centrifuge for 10min at 1000 rpm.

⑥ Repeat step 5 twice.

⑦ Preparation of cell suspension: aspirate the supernatant and add an appropriate amount of fixed solution to prepare a cell suspension with appropriate concentration. Preparation: after gently beating the cell suspension with a pipette, suck a small amount, drop it onto a clean fat free glass slide soaked in ethanol, drop 1-2 drops on each slide, and aging at 56℃ for 0.5h.

⑧ The prepared slides can be stored in 4℃ refrigerator or -20℃ refrigerator for about 1-4 weeks.

3. Slide pretreatment procedure

① At room temperature, place the slide in 2 × SSC (pH 7.0) solution, rinse twice for 5min each time.

② The slides were dehydrated in 70% ethanol, 85% ethanol and 100% ethanol for 2min respectively, and the slides were naturally dried.

③ The hybridization experiment was carried out according to the hybridization procedure.

4. 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 88°C for 2 minutes (the hybridizer should be preheated to 88°C) and hybridize at 45°C for 2 to 16 hours.

5. Washing

The following operations should be performed in a darkroom.

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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 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.

6. 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.

7. 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 40x, 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 were randomly counted and the number of normal signal cells and abnormal signal cells were counted. Each cell was counted once. Only cells with hybridization signals (both color signals) were counted. Cells with no signal or only a single color signal were not counted. Cells with weak signal or too diffuse signal were 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 bone marrow samples from patients with non-B cell chronic lymphoproliferative diseases or normal people as negative controls.
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

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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 500 cells to judge the final result.

Taking Table 2 as an example, if the percentage of abnormal signal cells in the FISH test results of the samples to be tested is greater than 3.9%, i.e. the abnormal threshold (e.g., 8%), it is judged that the patient has Ig λ (IGL, lambda) gene rearrangement; If the percentage of abnormal signal cells in the FISH test results of the sample to be tested is less than 3.9%, i.e. the abnormal threshold (e.g. 2%), it is judged that the patient has no Ig λ (IGL, lambda) gene rearrangement.

[Limitations of inspection methods]

This kit is only for Ig λ (IGL, lambda) gene rearrangement cannot be used as the only basis for clinical diagnosis, and it needs to be comprehensively evaluated on the basis of the patient's 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 22 of 50 cells in metaphase were analyzed, and at least 98 chromosomes 22 showed one orange red fluorescence signal and one green fluorescence signal.
3. Specificity: 100 chromosomes 22 of 50 cells in metaphase division phase were analyzed, and at least 98 chromosomes 22 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, but also limited by enzyme digestion time, hybridization temperature and time, operating environment and limitations of current molecular biology technology, which may lead to wrong 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.

[Manual Approval date & Revision date]

V1. 0: Approval date: November 02, 2018.
V1. 4: Revision date: December 07, 2021.