BCR/ABL gene fusion detection kit (ES)
Instructions Manual

[Product Name] BCR/ABL gene fusion detection kit (ES)

[Package Specifications] 10 Tests/box

[Intended Usage]

This kit is mainly used for the detection of BCR/ABL gene fusion in vitro. The test samples are bone marrow cells suspected or diagnosed with leukemia patients (clinical or post-treatment patients) by clinical routine examination and used only for auxiliary diagnosis of the patient's molecular typing.

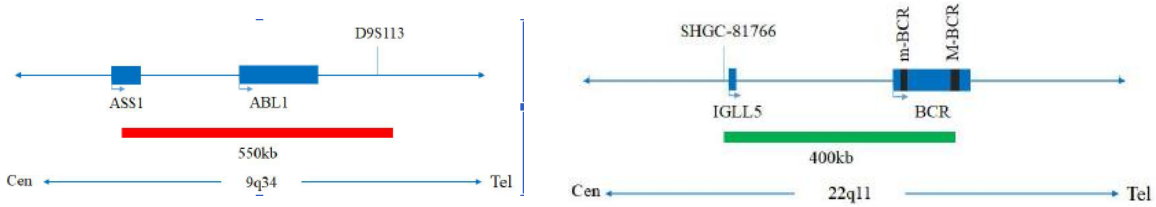
Leukemia is a kind of malignant clonal disease of hematopoietic stem cells. Clonal leukemic cells proliferate and accumulate in bone marrow and other hematopoietic tissues, and infiltrate other non-hematopoietic tissues and organs, because of an uncontrolled proliferation, differentiation and apoptosis, and inhibition of the normal hematopoietic function. BCR/ABL gene is a common cytogenetic anomaly in patients with chronic myelocytic leukemia (CML). The BCR/ABL fusion gene can be found in 90% of CML patients, and the prognosis of the patients with BCR/ABL gene is poor.

This kit was validated against the BCR/ABL gene fusion detection performance only, and was not combined with the drug for clinical validation. This kit is only suitable for the detection of BCR/ABL gene fusion status, the test results are for clinical reference only and should not be used as the only basis for diagnosis. The clinician should make comprehensive judgment on the test results in combination with other clinical indicators.

[Detection Principle]

Fluorescence in situ hybridization is a technique for direct detection in vitro of specific nucleic acids in cells. According to the principle of complementary bases pairing, a specific probe is complementary to a target sequence within the cell. The probe and target sequence can be clearly observed under fluorescence microscope and under appropriate excitation light, due to the probe fluorescence.

The kit uses orange fluorescein-labeled ABL probe and green fluorescein-labeled BCR probe. By in situ hybridization technique, the two probes bind to the target detection site. Normally (if BCR/ABL gene have not fused), two orange red signals and two green signals are shown under fluorescence microscope.When there is fusion, green and orange signals form by recombination a yellow fusion signal.



[Product Main Components]

The kit consists of ABL orange probe and BCR green probe, as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
BCR/ABL dual color probe	100μl/Tube	1	ABL orange probe, BCR green probe

[Storage conditions & Validity]

Keep sealed away from light at -20ºC±5ºC. The product is valid for 12 months. Avoid unnecessary repeated freezing and thawing that should not exceed 10 times. After opening, 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. See the label of the kit for the production date and expiration date.

[Applicable Instruments]

Fluorescence microscopic imaging system, including fluorescence microscope and filter set suitable for DAPI (367/452), green (495/517) and orange (547/565).

[Sample Requirements]

1. Applicable specimen types: Fresh specimen that have not been fixed stored at 4°C for less than 24 hours; Cell suspensions after fixation stored at -20°C for less than 6 months; Prepared cell slides stored at -20°C for less than 1 month.
2. When specimen are stored at too high or too low a temperature (eg, frozen), the specimen will not be used for testing and should be discarded.
3. If the cell suspension is excessively volatile or contaminated during storage, the sample should be discarded.

[Testing Method]

1. Related reagents

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

20×SSC, pH 5.3±0.2

Sodium chloride	176g
Sodium citrate	88g

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

NP-40	0.6mL
20xSSC	4mL

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.

⑤Fixation 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.

⑥ 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.⑦ Diamidinyl phenylindole (DAPI) counterstain

Use commercially available anti-quenching DAPI counterstain.

2. Sample collection and Slides preparation

① Sample collection: Take anticoagulant cells sample.

② Cells harvest: The cells sample is pipetted to the tip of centrifuge tube and centrifuge at 1000rpm for 10 minutes to remove the supernatant.

③ Low permeability: Add 0.075mol/L KCL solution (6~8mL) pre-warmed at 37°C, mix with a pipette, and store in an incubator at 37°C for 20 to 30 minutes.

④: Pre-fixation: Add 2mL of 3:1 methanol, glacial acetic acid fixative solution and mix evenly. Centrifuge at 1000rpm for 10min.

⑤Fixation:Aspirate the supernatant; add freshly prepared 5mL of 3:1 methanol - glacial acetic acid fixative solution, mix evenly, fix for 10 min, centrifuge at 1000 rpm for 10 min.

⑥ Repeat step ⑤ until cell pellets are precipitated, washed and cleaned.

⑦ Cells suspension preparation: Pipet the supernatant and add the appropriate amount of fixative solution to prepare the appropriate cells suspension concentration.

⑧ Slides preparation: Pipet 3-5μl of cells suspension drop onto the slides, put at 56°C for 0.5-2 hours.

⑨ The prepared slide can be stored in a refrigerator at 4°C or in a refrigerator at -20°C for about 1 to 4 weeks.

3. Slides pretreatment

① At room temperature, rinse the glass slides twice with SSC (pH 7.0) solution for 5min each time.

②Place the glass slides in 70% ethanol, 85% ethanol and 100% ethanol and dry for 2 minutes.

4. Denaturation and Hybridization

The following operations should be performed in a darkroom.

① Take out BCR/ABL 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.

① Take out the hybridized glass slides, remove the rubber on the coverslip and immediately place the slides into 2xSSC for 5 seconds, and gently remove the coverslip.Place the glass slides in 2xSSC at room temperature for 1 min.

② Remove and immerse the slides in a 0.3% NP-40/0.4×SSC solution preheated at 68℃ for 2 min.

③ Immerse the glass slides in deionized water at 37℃ for 1min, and dry naturally in the dark.

6. Counterstaining

The following operations should be performed in a darkroom.

Dip 10μL of DAPI counterstain into the hybridization area of the glass slides, immediately cover and then use the appropriate filter to observe the sections under the fluorescence microscope.

7. Preservation of slides after hybridization

After hybridization, washing and re staining, the slides can be sealed and stored in the dark at - 20 ℃± 5 ℃, and can be observed under normal microscope within 20 days.

8. FISH results observation

① Results observation method: put the counterstained glass slide under the fluorescence microscope, and first put it under the low power objective lens (10 ×） Confirm the cell area under the microscope; Go to 40 × Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100), The FISH results of nuclei were observed. During microscopic examination, the continuous irradiation time of a single visual field under the green channel and red channel shall be controlled within 40 minutes.

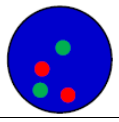
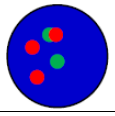
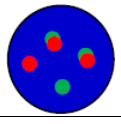
② Interpretable sample standard: the hybridization signal of the probe is bright and clear, the orange and green signals are easy to distinguish, the spontaneous fluorescence does not affect the signal count, and the number of countable cells is not less than 200.

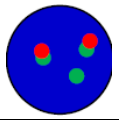
③ Countable cell standard: the cell distribution is reasonable, there is no overlap, DAPI counterstaining is clear, that is, the nuclear boundary is clear, and the number of orange, green signals or yellow signals formed by fusion in cells is ≥ 1.

④ Counting method: randomly count 200 cells in each sample, count the number of orange, green and yellow fusion signals in each nucleus, and calculate the ratio of cells showing abnormal cell signal mode (number of abnormal cells / number of counted cells) × 100%）。

Place the stained sections under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10×); under magnification objective (40×) a uniform cells distribution is observed; then the nucleus size uniformity, nuclear boundary integrity, DAPI staining uniformity, no nuclei overlapping, cells clear signal are observed in the high magnification objective (60x, 100x). Select randomly 200 cells at least and count the orange and green signals in the nuclei.

[interpretation of common signal types]

Signal type	Diagram pattern ● ABL ● BCR	Cells results determination
2 orange 2 green		Negative
2 orange1 green 1fusion		Positive
1 orange 1 green 1fusion (BCR at the main fracture point with ASS deletion)		Positive
1 orange1 green 2 fusion (BCR at the Secondary fracture point)		Positive

1 green 2 fusion (BCR at the Secondary fracture point with ASS deletion)		Positive

[Precautions]

1. Please read this manual carefully before testing. Operator should undergo professional technical training. Signal counting personnel must be able to observe orange and green signals.
2. When testing clinical samples, the test will not provide any test results when the hybridization signal is difficult to count and the sample is not sufficient for repeated retests. If the amount of cells is not sufficient for analysis, the test will not provide test results.
3. The DAPI dye used in this experiment are potentially toxic or carcinogenic and should be handled in a fume hood. Wear masks and gloves to avoid direct contact.
4. The results of this kit will be affected by various factors of the sample itself, as well as restrictions such as enzyme digestion time, hybridization temperature and time, operating environment, and limitations of current molecular biology techniques, which may result in erroneous interpretation results. User must understand the potential errors and accuracy limitations that may exist during the testing process.
5. All chemicals are potentially dangerous. Avoid direct contact. The used kits are clinical waste and should be properly disposed off.

[Basic information]

Name of registrant / manufacturer: Wuhan HealthCare Biotechnology Co., Ltd.

Address: Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.

Contact: 027-87570662

Name of after-sales service unit: Wuhan HealthCare Biotechnology Co., Ltd.

Contact: 18140559890

Production address: Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.

Production license No.: Hubei Food and Drug Supervision Equipment Production Xu No. 20170738

[approval date and modification date of the specification]

V1. 0 approval date: November 1, 2019

V1. 1 approval date: December 24, 2021

Manufacturer

Wuhan HealthCare Biotechnology Co., Ltd.

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