

## BCR/ABL (DF) Gene Fusion Probe Detection Kit

**[Product Name]** BCR/ABL (DF) Gene Fusion FISH Probe Detection Kit

**[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.

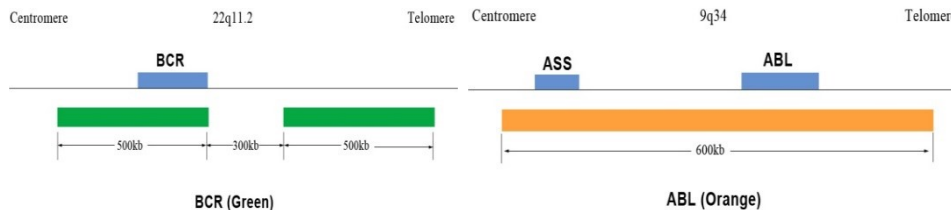
Leukemia is a kind of malignant clonal disease of hematopoietic stem cells. Clonal leukemic cells proliferate and accumulate in bone marrow and other hematopoietic tissues, and infiltrate other non-hematopoietic tissues and organs, because of an uncontrolled proliferation, differentiation and apoptosis, 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 -20°C±5°C. 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 2~8°C in dark. For long-term preservation after opening, keep the lid sealed at -20°C±5°C away from light. See the label of the kit for the production date and expiration date.

#### [Applicable Instruments]

1. Fluorescence microscopy imaging system includes fluorescence microscope and filter sets. The kit is labeled with orange fluorescein, and the filter set compatible with the fluorescent labeled dye should be selected.

Orange fluorescence: The maximum excitation wavelength is 552nm and the maximum emission wavelength is 576nm.

Green fluorescence: The maximum excitation wavelength is 496nm and the maximum emission wavelength is 520nm.

Fluorescence microscopy imaging system should use a microscope with orange and green channels. For monochromatic channel microscope, image synthesis analysis results should be used.

2. Automatic hybridization instrument: Strict temperature uniformity is required, and the temperature difference should be  $\leq 1^{\circ}\text{C}$ .

#### [Sample Requirements]

1. Applicable specimen types: Fresh specimen that have not been fixed stored at  $4^{\circ}\text{C}$  for less than 24 hours; Cell suspensions after fixation stored at  $-20^{\circ}\text{C}$  for less than 6 months; Prepared cell slides stored at  $-20^{\circ}\text{C}$  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

##### 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  $2\sim 8^{\circ}\text{C}$ , 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 20×SSC, 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  $2\sim 8^{\circ}\text{C}$ , 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-40/0.4×SSC 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  $2\sim 8^{\circ}\text{C}$ , the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

##### ⑤ 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.

##### 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 Diamidyl 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  $37^{\circ}\text{C}$ , mix with a pipette, and store in an incubator at  $37^{\circ}\text{C}$  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.

⑤ Fixation: 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 3-5 $\mu$ l 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.
- ② Place 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 10 $\mu$ l 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-40/0.4xSSC solution preheated at 68°C for 2 min.
- ③ Immerse the glass slides in deionized water at 37°C for 1min, and dry naturally in the dark.

### 6. Counterstaining

The following operations should be performed in a darkroom.

Dip 10 $\mu$ L 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°C $\pm$ 5°C, and can be observed under normal microscope within 20 days.



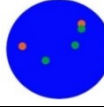
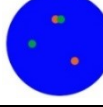
### 8. FISH results observation

- ① Results observation method: put the counterstained glass slide under the fluorescence microscope, and first put it under the low power objective lens (10x) Confirm the cell area under the microscope; Go to 40 $\times$  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  $\geq 1$ .
- 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)  $\times 100\%$ . Place the stained sections under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10 $\times$ ); under magnification objective (40 $\times$ ) 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 (60 $\times$ , 100 $\times$ ). Select randomly 200 cells at least and count the orange and green signals in the nuclei.

[Positive judgment value]

1. Common signal classification

Table 2 Dual-color for signal counting guide

Signal type	Diagram pattern ● ABL ● BCR	Cells results determination
Two orange red signals, two green signals (2R2G)		Negative
One orange signal, one green signal, two fusion signals (1R1G2F)		Positive
One orange signal, two green signals, one fusion signal (1R2G1F)		Positive
One orange signal, one green signal, one fusion signal (1R1G1F)		Positive

2. Threshold setting

Threshold should be set independently by each laboratory: Twenty human peripheral blood cell samples were randomly selected and processed according to sample processing requirements to prepare a deviant threshold reference slide. 200 cells were randomly counted in each reference slide. The number and percentage of cells that showed various types of positive cells were calculated, the mean and standard deviation of the statistical percentages were calculated.

- ① Calculation of the total cells percentage with the 1R1G1F signal type, the mean and standard deviation of the statistical percentage, and the deviant threshold is set as the mean + 3 times the standard deviation, recorded as the threshold A.
- ② Calculation of the total number cells percentage with other fusion signal types except 1R1G1F, the mean and standard deviation of the statistical percentage, and set the deviant threshold as the mean + 3 times the standard deviation, which is recorded as the threshold B. The threshold A is 13.5% and the threshold B is 4%, can be used as a reference. Due to differences in sample processing methods and the subjective nature of signal counts, the laboratory thresholds will be different. Each laboratory should establish thresholds strictly in accordance with the standard process set by the threshold.

[Interpretation of test results]

1. FISH results determination

After counting 200 cells, the number and percentage of various types of positive cells were calculated respectively,

- ① If the ratio of positive cells of 1r1g1f signal type > threshold a, it is judged as positive;
- ② Except for 1r1g1f signal type, the ratio of positive cells of other fusion signal types > threshold b, which is judged to be positive.
- ③ If the ratio of positive cells of 1r1g1f signal type is less than threshold a, it is judged as negative.
- ④ Except for 1r1g1f signal type, the ratio of positive cells of other fusion signal types < threshold b, which is interpreted as negative.
- ⑤ If the ratio of positive cells = threshold (a or b), increase the number of counts or analyze the whole film. If it is still less than or equal to the abnormal threshold, it will be interpreted as negative, otherwise it will be interpreted as positive.
- ⑥ If the ratio of positive cells is within  $\pm 3$  times the standard deviation of the mean value, the sample should be treated with caution.

2. Judgment of invalid experiment

- ① If the number of cells available for probe analysis is less than 200, this test shall be judged as invalid.
- ② If the fluorescence hybridization signal intensity or background available for analysis is not ideal or clear, which affects the judgment of results, this detection shall be judged as unreliable and treated as invalid experiment.

3. Common problems and treatment methods in the experiment

The factors affecting the test results and treatment methods in the experiment are shown in Table 3:

**Table 3 FAQ and solutions**

Question	Possible cause	Recommended solution
<b>Too strong background</b>	Slides were not cleaned properly before specimen's preparation.	Follow the recommended procedures for washing glass slides.
	Inadequate washing after hybridization.	Ensure that the washing solution is prepared according to the instructions, ensure the correct pH value and temperature of the washing solution, remove the coverslip and repeat the washing steps.
	Improper use of filter sets.	Replace the appropriate filter sets for observation and to weaken the background light.
	Improper hybridization conditions.	Ensure that the hybridization instrument temperature is 45°C.
	Low washing temperature.	Ensure that the solution temperature is at the washing slides required temperature.
<b>Too weak dye</b>	Too weak dye.	68°C 0.3%NP-40/0.4 × In SSC solution, shake for 10 ~ 20 seconds, remove the cover glass and soak for 2 minutes. Place the slide in deionized water at 37 °C and soak it for 1 minute. Dry the slide naturally in the dark and then re dye it.
	Obsolete dye agent or excessive illumination	Ensure that the dye agent is stored at -20°C and keep away from light. Make sure that the dye agent is valid.
<b>No signal or weak signal</b>	Specimen incomplete denaturation.	Ensure that the hybridization instrument temperature is at 88°C, and the hybridization instrument should be preheated at least 10min ahead of time.
	Improper pre-denaturation specimens' preparation.	Please refer to the above sample preparation related questions and answers.
	Probes and hybridization buffer improper mixture before usage.	Mix well the probe and the hybridization buffer, centrifuge briefly.
	Probe mixture on the slide dries too fast	Wash the coverslip in the washing solution.
	Bubbles formation under coverslips during hybridization.	When covering the coverslip, cover the surface of the probe mix and squeeze gently to allow the bubbles to escape.
	Inappropriate hybridization conditions.	Ensure to observe time and temperature specified for the hybridization; do not leave gaps in the rubber seals; adjust hybridization time as appropriate.
	Improper washing solution or washing conditions.	Ensure that the washing solution is prepared according to the product specification; Check that the washing solution temperature reaches the in the washing step specified temperature; Assure that the thermometer and the pH meter are correctly calibrated.
	Probe or specimen slides inadequate storage.	Ensure that the probe is stored in dark at -20°C. Place the unhybridized slides dry at -20°C for a long conservation or at room temperature for a short storage. Place the hybridized slides at -20°C and store in dark. The storage period should not exceed 6 months.
	Incorrect dye agent or too bright dye agent usage.	68°C 0.3%NP-40/0.4 × In SSC solution, shake for 10 ~ 20 seconds, remove the cover glass and soak for 2 minutes. Place the slide in deionized water at 37 °C and soak it for 1 minute. Dry the slide naturally in the dark and then re dye it.
	The selected filter sets is unsuitable for observation.	Use the correct filter sets to observe the probe fluorescence. For details, please contact Wuhan HealthCare Biotechnology Co., Ltd. Technical Service Department.

**[Test Method Limitations]**

This kit is used for the detection of BCR/ABL fusion gene and cannot distinguish the major (M-BCR) and minor (M-BCR) fracture points. This kit is only applicable to the detection of BCR/ABL fusion gene status. The test results are only for clinical reference and cannot be used as the basis for diagnosis or treatment alone.

**[Product Performance Index]**



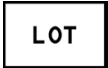




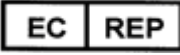



1. Appearance: the outer package of the kit is complete without damage, and the marks are complete and clear; The liquid reagent shall be clearly marked without leakage.

2. Fluorescence signal intensity: after the probe is effectively hybridized with the karyotype reference, it will send out fluorescence signals that can be recognized by the naked eye under the fluorescence microscope.
3. Sensitivity: after the probe effectively hybridized with the karyotype reference, 100 chromosomes 22 of 50 cells in metaphase were analyzed, and at least 98 chromosomes 22 showed 1 green fluorescence signal; 100 chromosomes 9 of 50 cells in metaphase were analyzed, and at least 98 chromosomes 9 showed an orange fluorescence signal.
4. Specificity: after the probe effectively hybridized with the karyotype reference, 100 chromosomes 22 of 50 cells in metaphase were analyzed, and at least 98 chromosomes 22 showed a specific green fluorescence signal in the long arm target area; 100 chromosomes 9 of 50 cells in metaphase were analyzed, and at least 98 chromosomes 9 showed a specific orange fluorescence signal in the long arm target region.
5. Coincidence rate of negative and positive: five negative reference materials (bone marrow cells of leukemia patients with BCR/abl fusion gene negative clinical diagnosis) were detected and the fluorescence signals were analyzed. The results met the negative judgment criteria and were all judged to be negative. Five positive reference materials (bone marrow cells of leukemia patients with BCR/abl fusion gene positive clinical diagnosis) were detected and the fluorescence signals were analyzed. The results met the positive judgment criteria and were all judged to be positive.
6. Precision: the precision of this kit within batch, between batches, within operation, between operation, during the day, and between operators / film readers is less than 5%.
7. Interfering substance: heparin sodium as anticoagulant will not interfere with the test results.
8. Clinical trial: among the 200 samples tested by this kit and the comparison reagent at the same time, the positive coincidence rate, negative coincidence rate and overall coincidence rate of this kit were 100%, and the kappa value was 1.00 ( $P < 0.001$ ); In 1086 samples detected simultaneously by this kit and karyotype analysis, the positive coincidence rate of this kit was 96.39%, the negative coincidence rate was 99.13%, the overall coincidence rate was 98.71%, and the kappa value was 0.95 ( $P < 0.001$ ).

#### **[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.
6. This product is for clinical diagnosis and scientific research.

**[Explanation of Symbols]**

	Use by		In vitro diagnostic medical device
	Batch code		CE marking
	Manufacturer		Warning sign
	Catalogue number		European Authorised Representative
	Temperature limitation		Consult Instructions For Use
	Contains sufficient for <n> tests		

**[Reference]**

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**[Manuscript version and approval date]**

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