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Product Catalogue Number FP-170 **For clinical diagnosis and scientific research.**

CHIC2 Gene Deletion (PDGFRA Break Apart) Probe Detection Kit

[Product Name] CHIC2 Gene Deletion (PDGFRA Break Apart) Probe Detection Kit (Fluorescence In-Situ Hybridization Method).

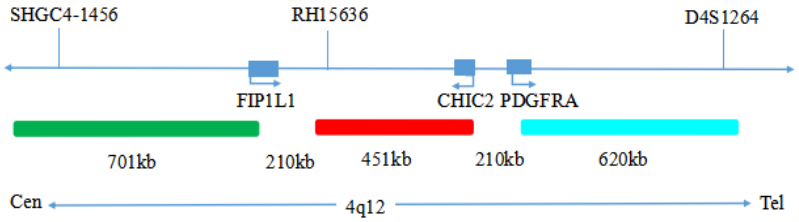
[Intended use]
The reagent carries out in situ hybridization staining on the basis of routine staining to provide doctors with auxiliary information for diagnosis. The test results are only for clinical reference and should not be used as the only basis for clinical diagnosis. Clinicians should comprehensively judge the test results in combination with the patient's condition, drug indications, treatment response and other laboratory test indicators.

[Detection principle]
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. Acco rding to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Due to the fluorescence o f the probe, the gene state of the hybrid probe and the target sequence can be clearly observed under the fluorescence microscope under the appropriate excitation light.

[Product Composition]
The kit consists of PDGFRA tricolor probes as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
PDGFRA tricolor probes	100μL/Tube	1	CHIC2 Orange probe, FIP1L1Green probe, PDGFRA Aqua probe



[Storage conditions & Validity]
The kit is transported under 0℃. Keep sealed away from light at -20℃±5℃. 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℃ in dark. For long-term preservation after opening, keep the lid sealed at -20℃± 5℃ away from light.

[Applicable Instruments]
Fluorescence microscopic imaging system, including fluorescence microscope and filter set suitable for DAPI (367/452), green (495/517), orange (547/565) and aqua (431/480).

[Sample Requirements]
1. Sample collection: take 1-3ml of bone marrow cells anticoagulated with heparin sodium.
2. Sample preservation: unfixed fresh bone marrow cell samples shall be stored at 2-8℃ for no more than 24 hours. After fixation, the cell suspension was stored at -20℃±5℃ for no more than 12 months. When the sample storage temperature is too high or too low, or the cell suspension is volatilized excessively or polluted during storage, the sample will not be used for detection.

[Related Reagents]

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 **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**

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.

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 **Diamidinyl phenylindole (DAPI) counterstain**

Use commercially available anti-quenching DAPI counterstain.

[Instructions]

1.Sample pretreatment of hybridization

① Sample collection: take 1-3ml of heparin sodium anticoagulant bone marrow cells.
② Cell harvesting: suck the uncultured bone marrow cells or the cultured bone marrow cell samples into a 15ml tip bottom centrifuge tube, centrifugate 500g for 5min, carefully suck and discard the supernatant, and leave about 500μl residual liquid to suspend the cells again.
③ Cell washing: add 5ml of 1×PBS buffer solution, blow and mix up the heavy suspension cell precipitation, centrifugate 500g for 5min, carefully suck and discard the supernatant, and leave about 500μl of residual solution to heavy suspension cell; repeat once.
④ Cell hypotonic: add 10ml of hypotonic solution to each tube (37°C warm bath in advance), and water bath at 37°C hypotonic for 20min.
⑤ Cell pre fixation: add 1ml (10% volume) of fixed solution to the cell suspension after hypotonic treatment, gently blow and mix, centrifugate 500g immediately for 5min, remove the supernatant, and leave about 500μl of residual solution for cell suspension.
⑥ Cell fixation: slowly add 10ml of the fixed solution to the cell suspension, leave it at room temperature for 10min to fix the cell, centrifugate 500g for 5min, and leave about 500μl of the residual solution to re suspend the cell; repeat once (or fix the cell several times until the cell is precipitated, washed and cleaned).
⑦ Preparation of cell suspension: after the last centrifugation of cell fixation, the supernatant is sucked off, and a proper amount of fixed solution is added to make cell suspension with appropriate concentration.
⑧ Preparation: take 3-10μl cell suspension drop to slide, aging at 56°C for 0.5h.
⑨

2. Slide pretreatment procedure

① Pretreatment: the slides were rinsed twice in 2×SSC solution at room temperature for 5min each time.
② Dehydration: the cell drops were placed in 70% ethanol, 85% ethanol and 100% ethanol for 2 minutes 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 22mm x22mm 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 88oC) and hybridize at 45°C for 2-16 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 soluti on 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.

5. Dyeing

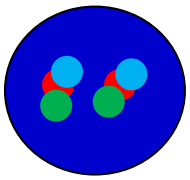
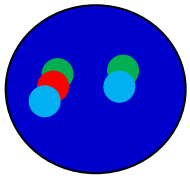
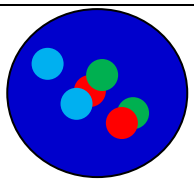
The following operations should be performed in a darkroom

10μl DAPI compound dye is dropped in the hybridization area of the glass slide and immediately covered. The suitable filter is s elected for glass slide observation under the fluorescence microscope.

6. FISH results observation

Place the counterstained glass slide under the fluorescence microscope, and first put it under t he low-power objective lens (10×) Confirm the cell area under the microscope; Go to 40 × Under the objective lens, a position with uniform cell distribution, complet e nuclear boundary, uniform DAPI staining and no overlapping of nuclei was found; Then i n the high-power objective (100×) The fish results of nuclei are observed.

[Common Signal Type Interpretation]

CHIC2 gene site signal	FIP1L1 gene site signal	PDGFRA gene site signal
	**Negative**: 2 Tricolor fusions	
	**Positive**: 1 Tricolor fusion ; 1 Green-aqua fusion	
	**Positive**: • 1 Orange-green fusion • 1 Tricolor fusion • 1 Aqua	

[Limitations of test methods]

① The results of this kit will be affected by various factors of the sample itself, but also limited by hybridization temperature a nd time, operating environment and the limitations of current molecular biology technology, which may lead to wrong results.

② Users must understand the potential errors and accuracy limitations that may exist in the detection process.

[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.

[Information, Inquiries, Support]
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