# 12p Probe Detection Kit

**[Product Name]** 12p Probe Detection Kit (Fluorescence In Situ Hybridization Method).

**[Product Introduction]**

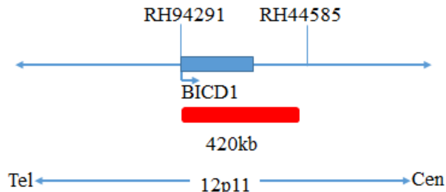
The kit uses orange fluorescein-labeled 12p11 probe and green fluorescein-labeled CEP12 probe to bind the 12p11/CEP12 probe to the target detection site by in situ hybridization.

**[Product Composition]**

The kit consists of 12p11/CEP12 dual-color probe, as shown in Table 1.

**Table 1: Kit composition**

| Component name | Specifications | Quantity | Main components |
|---|---|---|---|
| 12p11/CEP12 dual-color probe | 100μL/Tube | 1 | 12p11 orange probe；CEP12 green probe |




**[Storage conditions & Validity]**

Keep sealed away from light at -20ºC±5ºC, and the validity period is 12 months.
After the cover is opened, it can be sealed and stored in 2~8℃ away from light within 24 hours. After the cover is opened, it should be sealed and stored in -20±5℃ away from light for a long time. Transport with temperature below 0ºC.

**[Applicable Instruments]**

Fluorescence microscopy imaging systems, including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

**[Sample Requirements]**

**Cell sample:**

1. Take 1-3ml of heparin sodium anticoagulant bone marrow cells.
2. Sample preservation: Fresh bone marrow specimen without fixation (preserved at 2-8ºC for no more than 24 hours). After fixation, the cell suspension can be preserved at -20±5ºC for no more than 12 months; the prepared cell slide can be preserved at -20±5ºC for no more than 1 month. When the storage temperature of the sample is too high or too low, the cell suspension is volatilized excessively or polluted, the sample cannot be used for detection.

**Tissue sample:**

1. Applicable specimen types: Paraffin-embedded specimens from surgical excision or biopsy.
2. The tissue should be fixed with 4% neutral formaldehyde solution within 1 hour after isolation. After tissue fixation, it is routinely dehydrated and embedded in paraffin.

**[Preparation of relevant 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 processing before hybridization:**

**Cell sample:**

① Sample collection: take 1-3ml of heparin sodium anticoagulant bone marrow cells.

② Cell harvesting: the uncultured marrow cells or the cultured marrow cell samples were aspirated to a 15mL centrifuged tube at the bottom of the tip, and centrifuged at 500g for 5min. The supernatant was carefully aspirated and discarded, leaving about 500μL of residual liquid to suspend the cells again.

③ Cell washing: add 5ml of 1xPBS 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 The slides were rinsed twice in 2xSSC solution at room temperature for 5min each time.

⑩ Dehydration: the cell drops were placed in 70% ethanol, 85% ethanol and 100% ethanol for 2 minutes respectively and then dried naturally.

**Tissue sample:**

Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.

Dewaxing: According to the customer laboratory protocol (Commonly with Xylene for 15min).

Hydration: Take out the slides and put them respectively into 100%, 85% and 70% EtOH at room temperature for 3 minutes each.

Take out the slides, and immerse them in deionized water for 3 minutes. Remove the excess of water on the slides by air-drying.

Permeation: Immerse the slides in deionized water at 100°C and boil continuously for 20-40 minutes (Conventional 20min). Remove the excess of water on the slides by air-drying.

Digestion: Protease enzymic digestion at 37°C for 10-40 minutes. Mix the protease work buffer (50mmol HCl) and the 10x protease solution (Pepsin concentration 5%) in a proportion of 9:1 to prepare the enzymatic digestion solution.

Washing: Wash with 2xSSC at room temperature for 5 minutes.

Dehydration: Take out the slides and dehydrate in 70%, 85%, and 100% gradient ethanol at room temperature for 2 minutes each time. Remove the excess of EtOH solution on the slides by air-drying.

**2. Denaturation and Hybridization**

The following operations need to be carried out in the darkroom.

**Cell sample:**

① Take out the probe, leave it at room temperature for 5min, turn it upside down with force, mix it well, and then centrifuge it for a short time (no vortex instrument vibration). Take 10μL of it and drop it into the cell drop hybridization area, immediately cover the cover glass of 22mm × 22mm. The probe should be evenly expanded under the cover glass without bubbles, and seal the edge with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results in the hybridization process).

② The cell drops were placed on the hybridizer and denatured at 88°C for 2min (the hybridizer should be preheated to 88°C) and hybridized at 45°C for 2 to 16 hours.

**Tissue sample:**

① Take out the probe, let it stand at room temperature for 5min, turn it upside down with force, fully mix the probe, and then centrifuge briefly (vortex instrument oscillation is prohibited), take 10μL was dropped on the hybridization area of cell drops and immediately covered with 22mm×22mm cover glass, the probe shall be evenly expanded under the cover glass without bubbles, and the edge shall be sealed with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results during hybridization).

② Put the tissue sections on the hybridizer, CO denature at 85℃ for 5min (the hybridizer should be preheated to 85°C in advance), and hybridize at 42°C for 2-16h.

**3. Washing**

The following operations need to be carried out in the darkroom.

① Carefully remove the sealing glue around the cover glass with tweezers to avoid sticking or moving the cover glass, immerse the sample in 2xSSC for about 5S, take it out, gently push a corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers.

② Place the sample at 2xSSC 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 Take out the sample and immerse it in deionized water preheated at 37°C in advance for 1min; dry it naturally in the dark environment.

**4. Counterstaining**

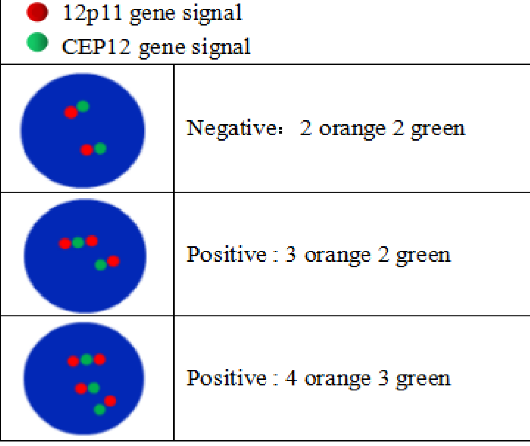
The following operations should be performed in a darkroom.

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

**5. FISH results observation**

Place the counterstained film under the fluorescence microscope, and first put it under the low-power objective lens (10x) Confirm the cell area under the microscope; Go to 40x Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100x) The FISH results of nuclei are observed.

**[Common Signal Type Interpretation]**




**[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 and 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.

**[Manuscript version and approval date]**

**Manual version:** V1. 3 reviewed on 21 September 2022.
**Manual version:** V1. 0 approval date 01 April 2021.