

## **CCND1(BCL1)/IGH Gene Fusion Probe Detection Kit (CW-233-1)**

### **Intended use**

The kit uses orange fluorescein-labeled CCND1 probe and green fluorescein-labeled IGH probe to bind CCND1/IGH probe to the target detection site by in situ hybridization.

### **Product composition**

The kit consists of CCND1/IGH dual color probe (100 $\mu$ L/Tube).

### **Storage condition**

Keep sealed away from light at  $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$ . 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  $2-8^{\circ}\text{C}$  in dark. For long-term preservation after opening, keep the lid sealed at  $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$  away from light. This kit is shipped below  $0^{\circ}\text{C}$ .

### **Applicable instruments**

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

### **Sample requirements**

#### ***Tissue samples:***

1. Applicable specimen type: paraffin embedded specimen of surgical resection or biopsy tissue.
2. The tissue in vitro should be fixed with 4% neutral formaldehyde fixative within 1 hour. After the tissue is fixed, it is often dehydrated and embedded in paraffin.

#### ***Cell samples:***

1. Applicable specimen type: fresh bone marrow specimen without fixation (stored at  $2-8^{\circ}\text{C}$  for no more than 24 hours).
2. Sample collection 1-3ml heparin sodium anticoagulant bone marrow cell samples.
3. Sample preservation: after fixation, the cell suspension can be stored at  $-20\pm 5^{\circ}\text{C}$  for no more than 12 months; the prepared cell slide can be stored at  $-20\pm 5^{\circ}\text{C}$  for no more than 1 month. When the storage temperature of the sample is too high or too low, or

the cell suspension is over volatilized or contaminated during storage, the sample can not be used for detection.

### **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. 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.

## **Sample pretreatment**

### ***Tissue samples***

It is recommended to use Cytowish's FISH pretreatment reagent (catalogue number CW-CT-FISH).

### ***Cell samples***

1. Sample collection: Take 1-3mL of anticoagulated bone marrow cell samples.
2. Cell harvesting: Place 3mL of anticoagulated peripheral blood in a 15 mL centrifuge tube, centrifuge at 500 g for 5 min, carefully discard the supernatant, and resuspend about 500µL of the residue.
3. Cell washing: Add 5 mL of 1×PBS buffer, mix and resuspend the cell pellet, centrifuge at 500 g for 5 min, carefully discard the supernatant, and resuspend the cells with about 500µL of the residue; repeat one time.
4. Cells hypotonicity: Add 10mL of hypotonic solution pre-warmed to 37°C and place in a water bath at 37°C for 20min.
5. Cells pre-fixation: Pre-fix the cells by adding 1mL (10% by volume) of fixative solution to the cell suspension after the completion of hypotonic osmosis. Gently pipette, mix and centrifuge for 5 min at 500 g, discard the supernatant, and resuspend about 500µL of the residue.
6. Cell fixation: Slowly add 10mL of fixative solution to the cell suspension at room temperature for 10 min, centrifuge at 500 g for 5 min, and resuspend the cells with about 500µL of the residue; repeat once (the cells may be fixed several times until the cells pellet is washed and cleaned).
7. Cell suspension preparation: Pipet the supernatant and add the appropriate amount of fixative solution to prepare the appropriate cell suspension concentration.
8. Slides preparation: Pipet 3-10µl of cell suspension drop onto the slides, put at 56°C for 30min.
9. At room temperature, rinse the glass slides twice with 2xSSC (pH 7.0) solution for 5min each time.
10. Place the glass slides in 70% ethanol, 85% ethanol and 100% ethanol and dry for 2 minutes.

## **Denaturation and hybridization**

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

***Tissue samples:***

1. Take the probe at room temperature for 5 minutes. Briefly centrifuge manually (do not use vortex or shaker instrument). 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 85°C for 5 minutes (the hybridizer should be preheated to 85°C) and hybridize at 42°C for 2 to 16 hours.

***Cell samples:***

1. Take out the 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.

**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.
2. Place the glass slides in 2xSSC at room temperature.
3. Remove and immerse the slides in a 0.3% NP-40/0.4xSSC solution preheated at 68°C for 2 min.
4. Immerse the glass slides in deionized water at 37°C for 1min, and dry naturally in the dark.

**Counterstaining**

The following operations should be performed in a darkroom.

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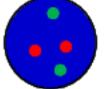
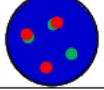
10 $\mu$ L DAPI compound dye is dropped in the hybridization area of the glass slide and immediately covered. The suitable filter is selected for glass slide observation under the fluorescence microscope.

### FISH results observation

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 (60x, 100x).

 CCND1 site signal	 IGH signal
	<b>Negative:</b> 2 Orange ; 2 Green
	<b>Positive:</b> 1 Orange ; 1 Green ; 2 fusions

### 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.

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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]

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