

## **RELA(11q13) Break Apart Probe Reagent (CW-229)**

### **Intended use**

This kit performs fluorescence in situ hybridization staining on the basis of conventional staining, and provides auxiliary information for diagnosis for physicians. The test results are for clinical reference only and should not be used as the sole basis for clinical diagnosis. Clinicians should make comprehensive judgments on the test results based on factors such as the patient's condition, drug indications, treatment response and other laboratory test indicators.

### **Product composition**

The kit consists of RELA dual-color probe (100 µL/Tube).

### **Storage condition**

Keep sealed away from light at  $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$ , and the validity period is 12 months. After the cover is opened, it can be sealed and stored in  $2\sim 8^{\circ}\text{C}$  away from light within 24 hours. After the cover is opened, it should be sealed and stored in  $-20\pm 5^{\circ}\text{C}$  away from light for a long time. Transport with temperature 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**

1. Applicable specimens types: Surgical resection or paraffin-embedded biopsy specimens.
2. Isolated tissue should be fixed in vitro with 4% neutral formaldehyde fixative within 1 hour. After tissue fixation, regular dehydration and paraffin embedding should be performed.

### **Sample processing before hybridization**

Baking: Slides heating at  $80^{\circ}\text{C}$  for 30min or  $65^{\circ}\text{C}$  for 2h or overnight.

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

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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.

### Denaturation and hybridization

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

1. Take the PD-L1/CSP9 probe at static room temperature for 5 minutes. Briefly centrifuge after manually mixing the probe (do not use vortex/swirl or shaker instrument/oscillator). 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 slide 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.

### 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.

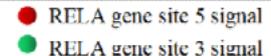
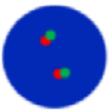
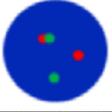
### Counterstaining

The following operations should be performed in a darkroom.

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.

### FISH results observation

Place the stained slide under a fluorescence microscope and the cells area is first confirmed under a low magnification objective (10x); under magnification objective (40x) 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 ( 100x).

	
	Negative: 2 fusion
	Positive : 1 orange 1 green 1 fusion

### 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.

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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]

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