

## **MYCN Gene Amplification Probe Detection Kit**

### **(CW-048)**

#### **Intended use**

This kit uses Orange Fluorescein labeled N-MYC probe and Green Fluorescein labeled LAF4, to combine N-MYC/LAF4 genes with the target site by in situ hybridization.

#### **Product composition**

The kit consists of N N-MYC/LAF4 dual color probe 100µl/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.

#### **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 specimen types: Paraffin-embedded specimens for surgical resection or biopsy.
2. Tissue should be fixed with 4% neutral formaldehyde fixation solution within 1 hour after ex vivo, and the tissue should be fixed by conventional dehydration and paraffin embedding.

#### **Sample treatment before hybridization**

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

#### **Denaturation and hybridization**

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

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 85°C for 5min (the hybridizer should be preheated to 85°C) and hybridized at 42°C for 2-16 hours

### **Washing**

The following operations should be performed in a 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 sample and immerse it in 0.3%NP-40/0.4xSSC solution preheated at 68°C 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 place.

### **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 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×); under magnification objective (40×) 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).

|  N-MYC signal |  LAF4 signal |
|------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
|               | Negative: 2 Orange ; 2 Green<br>(2R ; 2G)                                                     |
|               | Positive: n Orange ; 2 Green (n≥4)<br>(nR ; 2G, n≥4)                                          |
|               |                                                                                               |

## 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: [V1.0](#)

Approval date: [03 November 2019](#)