

CDK4(12q13)/CEP12 Probe Detection Kit (CW-149)

Intended use

This kit uses Orange fluorescein labeled CDK4 probe and Green fluorescein labeled CEP12, to combine CDK4/CEP12 genes with the target site by in situ hybridization.

Product composition

The kit consists of CDK4/CEP12 dual color probe (100µL/Tube).

Storage condition

This kit is shipped below 0°C. Keep sealed away from light at -20°C± 5°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°C in dark. For long-term preservation after opening, keep the lid sealed at -20°C± 5°C away from light.

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

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.

Denaturation and hybridization

The following operations should be carried out in the dark room.

1. Take the probe at 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).

- 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.

- Take out the hybridized glass slides, remove the rubber on the coverslip and immediately immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.
- Place the slides in a 2xSSC at room temperature for 1 min.
- Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution and wash for 2min.
- Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

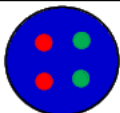
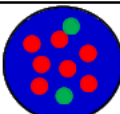
5. Counterstaining

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 slides under a fluorescence microscope and confirm the cells area under a low magnification objective (10x). Under magnification objective (40x) a uniform cells distribution is observed. Then the nuclei FISH results are observed under the high magnification objective (100x).

 CDK4 signal	 CEP12 signal
	Negative: 2 Orange ; 2 Green [2R-2G]
	Positive: n Orange (n≥4); 2 Green [nR(n≥4)-2G]

CDK4: Orange-red (R) pattern; CEP12: Green (G) pattern

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**

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