

CDK4(12q13)/CEP12 Probe Detection Kit

[Product Name] CDK4(12q13)/CEP12 Probe Detection Kit (Fluorescence In Situ Hybridization Method).

[Product Introduction]

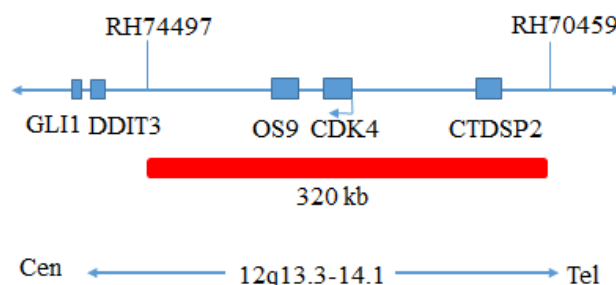
This kit uses Orange fluorescein labeled CDK4 probe and Green fluorescein labeled CEP12, to combine CDK4/CEP12 genes with the target site by in situ hybridization.

[Product Main Components]

The kit consists of CDK4/CEP12 dual color probe as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
CDK4/CEP12 dual color probe	100μL/Tube	1	CDK4 Orange probe ; CEP12 Green probe



[Storage conditions & Validity]

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]

- Applicable specimen types:** Paraffin-embedded specimens for surgical resection or biopsy.
- 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.

[Testing Method]

1. Sample Pretreatment

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd.'s "FISH Pretreatment Reagent Kit" (Cat.# CL-003) for pretreatment.

2. Denaturation and Hybridization

The following operations should be performed in a darkroom.

- ① Take the probe at room temperature for 5 minutes. Briefly centrifuge manually (do not use vortex or shaker instrument). Take 10 μ 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 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.

3. 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 for 1 min.
- 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.

4. Counterstaining



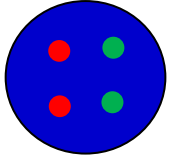
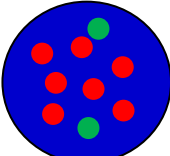
The following operations should be performed in a darkroom

10 μ 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.

5. 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).

[Common Signal Type Interpretation]

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

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

Test Method Limitations

- 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 limitations of current molecular biology technology, which may lead to erroneous results.
- ② The user must understand the potential errors and accuracy limitations that may exist in the detection process.

[Precautions]

1. This product is for research use only.
2. Please read this manual carefully before testing. The testing personnel should undergo professional technical training. The signal counter personnel must be able to observe and distinguish the orange-red and green signals.
3. The test will not provide any results when testing clinical samples it is difficult to count the hybridization signal and the sample is not enough to repeat the test, or the amount of cells is not enough for analysis.

4. The DAPI counterstaining agent used in this experiment is potentially toxic or carcinogenic. It must be operated in a fume hood. Inhalation and direct contact should be avoided by wearing the appropriate masks and gloves.
5. All chemicals are potentially dangerous. Avoid direct contact and wastes should be disposed off properly.

[Manuscript version and approval date]

Manual version: V1.0

Approval date: 14 November 2019
