

Product Catalogue Number FP015 For Research Use Only – RUO

MYC (8q24) Gene Amplification Probe Detection Kit

[Product Name] MYC(8q24) Gene Amplification Probe Detection Kit (Fluorescence In Situ Hybridization Method).

[Product Intended Use]

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

[Detection Principle]

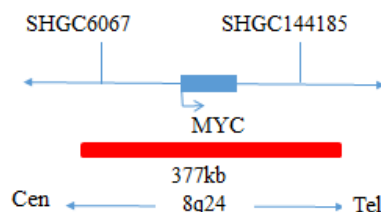
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of complementary base pairing, a specific probe is complementary to the target sequence in the cell. Because the probe is fluorescent, the hybridization probe and the target sequence can be clearly observed under a fluorescence microscope under the appropriate excitation light and the genetic status is observed.

[Product Composition]

The kit consists of C-MYC/CEP8 dual-color probes as shown in Table 1.

Table 1 Kit composition

Component name	Cat.#	Specifications	Quantity	Main components
C-MYC/CEP8 dual color probe	FP-015	100μL/Tube	1	C-MYC Orange probe CEP8 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]

1. Applicable specimens' types: Surgical excision or paraffin-embedded biopsy specimens.
2. The tissue should be fixed with 4% neutral formaldehyde fixation solution within 1 hour after in vitro. After tissue fixation, it should be regularly dehydrated and embedded in paraffin.

[Testing Method]

1. Pretreatment

Recommended to use the FISH pretreatment reagent of Wuhan HealthCare Biotechnology Co., Ltd. (Cat.# CL-003).

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 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 88°C for 2 minutes (the hybridizer should be preheated to 88°C) and hybridize at 45°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

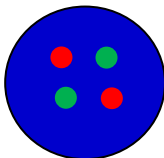
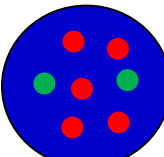
The following operations should be performed in a darkroom

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.

5. FISH results observation

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

[Common Signal Type Interpretation]

● MYC signal	● CEP signal
	Negative: 2 fusions (Negative: 2F)
	Positive: 2 Green ; n Orange (n≥3) [Positive: 2G-nR (n≥3)]

MYC: Orange-red (R) pattern; CEP8: 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 usage 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: V1.2 reviewed on 07 December 2021

Approval date: 29 October 2019
