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Product Cat. No.: GBS-015

For Research Use Only.

MYC(8q24) gene amplification probe reagent Instructions Manual

[Product Name] MYC(8q24) gene amplification probe reagent.
[Package Specifications] 10 Tests/box.

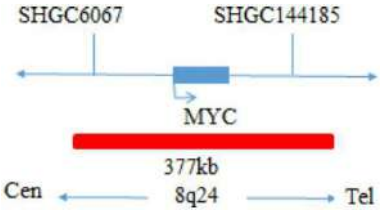
[Intended use]
The reagent carries out in situ hybridization staining on the basis of routine staining to provide doctors with auxiliary information for diagnosis. The test results are only for clinical reference and should not be used as the only basis for clinical diagnosis. Clinicians should comprehensively judge the test results in combination with 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. According to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Due to the fluorescence of the probe, the gene state of the hybrid probe and the target sequence can be clearly observed under the fluorescence microscope under the appropriate excitation light.

[Product Composition]
The kit consists of C-MYC/CEP8 dual color probe as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
C-MYC/CEP8 dual color probe	100μL/Tube	1	C-MYC Orange probe CEP8 Green probe



[Storage conditions & Validity]
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. The kit is transported below 0°C.

[Applicable Instruments]
Fluorescence microscopy imaging systems, including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

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[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. After tissue fixation, it should be regularly dehydrated and embedded in paraffin.

[Test method)

1. Pretreatment

Baking: Slides heating at 80°C for 30min or 65°C for 2h or overnight.

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

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 100oC 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.

2 . Denaturation & Hybridization

The following operations should be performed in a darkroom.

① Take the probe at static room temperature for 5 minutes. Briefly centrifuge (1-2s) 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 2mmx22mm 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 immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.

② Place the slides in a 2×SSC at room temperature for 1 min.

③ Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution and wash for 2min. (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2).

④ Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

4. 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.

5 . FISH results observation

Place the stained sections 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 (60x, 100x).

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[Common Signal Type Interpretation]

MYC CEP8	
	Negative:2 orange 2 green
	Positive :n orange 2 green (n ≥3)

[Limitations of test methods]

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

[Precautions]

① The results of this kit can be affected by various factors of the sample itself, as well as being limited by the temperature and hybridization time, the operating environment and limitations of current molecular biology techniques, which may lead to erroneous results.
② The user must understand the limitations of potential errors and inaccuracies that may exist in the testing process.