Wuhan HealthCare Biotechnology Co., Ltd.
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For Professional Use

MYCN gene amplification probe reagent Instructions Manual

[Product Name] MYCN gene amplification probe reagent

[Product introduction]

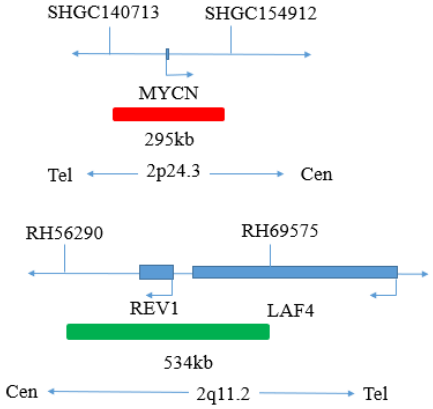
The kit uses orange fluorescein labeled N-MYC orange probe and green fluorescein labeled LAF4 green probe. N-MYC/LAF4 probe can be combined with the target detection site by in situ hybridization.

[Product Main Components]

The kit consists of N-MYC/LAF4 dual color probe as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
N-MYC/LAF4 dual color probe	100μL/Tube	1	N-MYC orange probe、LAF4 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 microscopic imaging system, including fluorescence microscope and filter set suitable for DAPI (367/452), green (495/ 517) and orange (547/565).

[Sample Requirements]

1. Applicable specimen type: paraffin embedded specimen of surgical resection or biopsy tissue.

2. The tissue in vitro should be fixed with 4% neutral formaldehyde fixative within 1 hour. After the tissue is fixed, it is often dehydrated and embedded in paraffin.

[Instruction]

1. Hybridization pretreatment

Recommended to use the "FISH Pretreatment Reagent" of Wuhan HealthCare Biotechnology Co., Ltd. for pretreatment.

2. Denaturing hybridization

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

① Take out the probe, let it stand at room temperature for 5min, turn it upside down with force, mix the probe well, centrifuge it briefly (do not vibrate with vortex apparatus), drop 10μl into the hybridization area of the cell drop, cover the 22mm×22mm cover glass immediately, the probe should be evenly spread under the cover glass without bubbles, and seal the edge with rubber (the edge sealing must be thorough to prevent the dry slide from affecting the test results in the hybridization process).

②Place the glass slide in the hybridization instrument, denature at 85°C for 5 min (the hybridizer should be preheated to 85°C) and hybridized at 42°C for 2-16h.

3. Washing

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

① Carefully tear off the adhesive around the cover glass with tweezers to avoid sticking off or moving the cover glass. Immerse the cell drop into 2xSSC for about 5s, and take it out. Gently push one corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers;

② The cells were placed at 2xSSC room temperature for 1min;

③ 3% NP-40/0.4 xSSC solution preheated at 68℃for 2min;

④ The sides were immersed in deionized water preheated at 37℃ for 1min, and then dried naturally in the dark.

4. Counterstaining

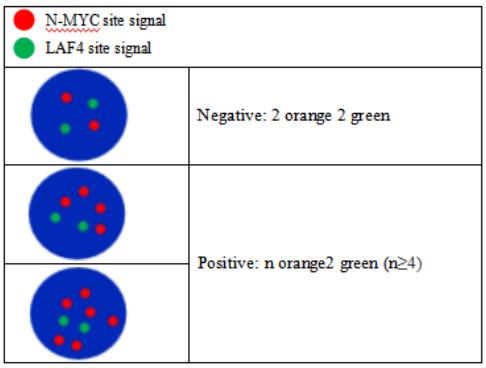
The following operations should be performed in a darkroom

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 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 (100x).

[Interpretation of common signal types]



[Precautions]

① This product cannot be used for clinical diagnosis, but only for scientific research.

② 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.

③ Users must understand the potential errors and accuracy limitations that may exist in the detection process.

④ All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed of.

[Basic information]

Name of registrant / manufacturer: Wuhan HealthCare Biotechnology Co., Ltd.

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[approval date and modification date of the specification]

V1. 0 approval date: November 3, 2019

V1. 4 approval date: December 7, 2021