

Wuhan HealthCare Biotechnology Co., Ltd.

Floor 1-4, Building #8, Optics Valley Precision Medicine Industry Base Phase I, #9 Gaokeyuan 3rd Road, East Lake High-Tech Zone, Wuhan City, Hubei Province, People's Republic of China.

For Professional Use

1p/19q deletion probe reagent Instructions Manual

[Product Name] 1p/19q deletion probe reagent

[Package Specifications] 5 Tests/box 10 Tests/box

[Product Introduction]

This kit uses orange fluorescent probes 1p36 and 19q13, green fluorescent probes 1q25 and 19p13 to bind 1p/19q probe to the target detection site by in situ hybridization.

[Product Main Components]

The kit consists of 1p36/1q25 dual-color probe and 19q13/19p13 dual-color probe as shown in Table 1.

Table 1 Kit composition					
Package Specifications	Component name	Specifications	Quantity	Main components	
5 Tests/box	1p36/1q25 dual color probe	50µl/Tube	1	1p36 orange probe 1q25 green probe	
	19q13/19p13dual color probe	50µl/Tube	1	19q13 orange probe 19p13 green probe	
10 Tests/box	1p36/1q25 dual color probe	100µl/Tube	1	1p36 orange probe 1q25 green probe	
	19q13/19p13 dual color probe	100µl/Tube	1	19q13 orange probe 19p13 green probe	
SHGC100402 RH78144 RH91552 RH39180 ARHGEF16 TP73 ZNF541 GLTSCR1 CRX				GC81940 RH14254	
	435kb Tel	→Cen Cen	1 «	315kb 19q13.3 — Tel	
SHGC57465 RH104509 ANGPTL1 ABL2 SOAT1 SHGC105252 RH55757 RH26548 RH55987 RH16594 ZNF44 ZNF709 MAN2B1 477kb					

[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 should be shipped below 0°C.

→ Te1

Te1

19p13.2 -

Cen

[Applicable Instruments]

Fluorescence microscopy imaging system 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 from surgical excision or biopsy.

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2. The tissue should be fixed with 4% neutral formaldehyde solution within 1 hour after isolation. After tissue fixation, it is routinely dehydrated and embedded in paraffin.

[Instructions]

1. Pre-hybridization treatment

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

2. Denaturation and Hybridization

The following operations should be performed in a darkroom.

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

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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 $2 \times SSC$ 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.

(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 counterstained film under the fluorescence microscope, and first put it under the low-power objective lens $(10 \times)$ Confirm the cell area under the microscope; Go to 40× Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective $(100 \times)$ The FISH results of nuclei were observed.

[Common Signal Type Interpretation]

1p36 and 19q13 signal	1q25 and 19p13 signal
	Negative: 2 Orange-red (2R) ; 2 Green (2G)
	Positive: 1 Orange-red (1R) ; 2 Green (2G)1p36/1q25 point-out, 1p36 missing.
	Positive: 1 Orange-red (1R) ; 2 Green (2G) 19p13/19q13 point- out, 19q13 missing.

[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]

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Manufacturer

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