FKHR gene break apart probe reagent Instructions Manual

[Product Name] FKHR gene break apart probe reagent

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

[Detection Principle]

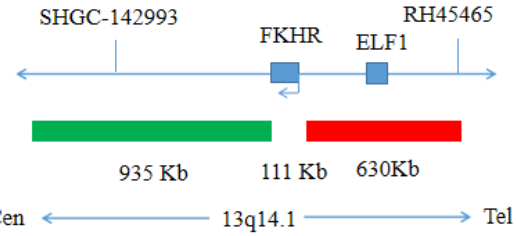
This kit uses orange-red (Orange) fluorescein and green (Green) fluorescein to label the FKHR probe, and the FKHR probe can be bound to the target detection site by in situ hybridization technology.

[Product Main Components]

The kit consists of FKHR dual-color probe, as shown in Table 1.

Table 1 Kit composition

Package Specifications	Component name	Specifications	Quantity	Main components
5 Tests/box	FKHR dual-color probe	50μl/Tube	1	FKHR Orange probe, FKHR Green probe
10 Tests/box	FKHR dual-color probe	100μl/Tube	1	FKHR Orange probe, FKHR Green probe



[Storage conditions & Validity]

Keep sealed away from light at -20°C±5°C, and the validity period is 12 months.

After the cover is opened, it can be sealed and stored in 2~8°C away from light within 24 hours. After the cover is opened, it should be sealed and stored in -20±5°C away from light for a long time. Transport with temperature 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).

[Sample Requirements]

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

2. Tissue should be fixed with 4% neutral formaldehyde fixation solution within 1 hour after in vitro, and the tissue should be fixed by conventional dehydration and paraffin embedding.

[Instructions]

1. Pre-hybridization or Pretreatment

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 need to be carried out in the darkroom.

① Take out the probe, leave it at room temperature for 5min, turn it upside down with force, mix it well, and then centrifuge it for a short time (no vortex instrument vibration). Take 10μL of it and drop it into the cell drop hybridization area, immediately cover the cover glass of 22mm × 22mm. The probe should be evenly expanded under the cover glass without bubbles, and seal the edge with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results in the hybridization process).

② The cell drops were placed on the hybridizer and denatured at 85°C for 5 min (the hybridizer should be preheated to 85°C) and hybridized at 42°C for 2-16 hours.

3. Washing

The following operations need to be carried out in the darkroom.

① Carefully remove the sealing glue around the cover glass with tweezers to avoid sticking or moving the cover glass, immerse the sample in 2xSSC for about 5S, take it out, gently push a corner of the cover glass to the edge of the slide with tweezers, and gently remove the cover glass with tweezers;

② Place the sample at 2xSSC room temperature for 1 min;

③ Take out the sample and immerse it in 0.3%NP-40/0.4xSSC solution preheated at 68°C for 2min;

④ Take out the sample and immerse it in deionized water preheated at 37°C in advance for 1min; dry it naturally in the dark place.

4. Counterstaining

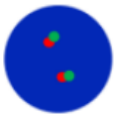
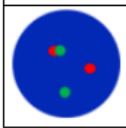
The following operations should be performed in a darkroom

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 counterstained film under the fluorescence microscope, and first put it under the low-power objective lens (10 ×) 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×) The FISH results of nuclei were observed.

[Common Signal Type Interpretation]

FKHR gene site 5 signal FKHR gene site 3 signal	
	Negative: 2 fusion
	Positive : 1 orange 1 green 1 fusion

[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: June 4, 2019

V1. 2 approval date: December 7, 2021