

Product Catalogue Number FP160 For Research Use Only – RUO

IRF4(6p25) Gene Break Apart Probe Detection Kit

[Product Name] IRF4(6p25) Gene Break Apart Probe Detection Kit (Fluorescence In Situ Hybridization Method).

[Intended Usage]

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

[Detection Principle]

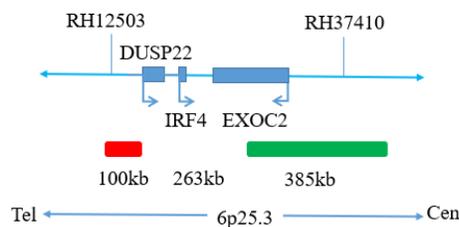
The kit is based on fluorescence in situ hybridization technology. A nucleic acid probe is labeled with fluorescein. The target gene is detected with homologous complementary to the nucleic acid probe used. Both after denaturation, annealing and renaturation, the hybrid of the target gene and the nucleic acid probe can be formed, and the qualitative, quantitative or relative positioning analysis of the gene to be measured under the microscope can be performed by the fluorescence detection system.

[Product Main Components]

The kit consists of IRF4 dual-color probes, as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
IRF4 dual color probe	100µl/Tube	1	IRF4 orange probe, IRF4 green probe, hybridization buffer



[Storage conditions & Validity]

Keep sealed away from light at -20°C±5°C, and the validity period is 20 months. For short conservation after opening, keep at +2°C to +8°C away from light within 24 hours. For long conservation after opening, keep at -20°C±5°C away from light for a long time. Transport under 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 from surgical excision or biopsy.
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.

[Testing Method]

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 10 μ l into the hybridization area of the cell drop, cover the 22mm \times 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).
- ② Place the glass slide in the hybridization instrument, denature at 85 $^{\circ}$ C for 5 min (the hybridizer should be preheated to 85 $^{\circ}$ C) and hybridized at 42 $^{\circ}$ 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 $^{\circ}$ C for 2min;
- ④ The sides were immersed in deionized water preheated at 37 $^{\circ}$ C for 1min, and then dried naturally in the dark.

4. Counterstaining

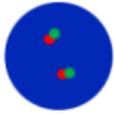
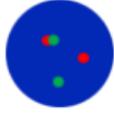
The following operations should be performed in a darkroom

10 μ l 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 \times); under magnification objective (40 \times) 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 (100 \times).

[Interpretation of common signal types]

● IRF4 gene site 5 signal ● IRF4 gene site 3 signal	
	Negative : 2 fusion
	Positive : 1 orange 1 green 1 fusion

[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 This product is for research use only.
- 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 and waste should be properly disposed off.

[Manuscript version and approval date]

Manual version: V1.2 reviewed on 07 December 2021

Approval date: 04 November 2019