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Product Cat. No.: GBS-056

For Research Use Only.

FOXO1(13q14) gene break apart probe reagent Instructions Manual

[Product Name] FOXO1(13q14) gene break apart probe reagent.
[Package Specifications] 10 Tests/box.

[Detection Principle]

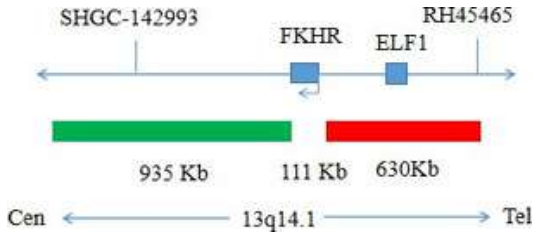
This kit uses orange-red (Orange) fluorescein and green (Green) fluorescein to label the FOXO1 probe, and the FOXO1 probe can be bound to the target detection site by in situ hybridization technology.

[Product Main Components]

The kit consists of FOXO1dual-color probe, as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
FOXO1 dual-color probe	100μL/Tube	1	FOXO1 Orange probe ; FOXO1 Green probe



[Storage conditions & Validity] Keep sealed away from light at -20℃±5℃, and the validity period is 12 months. After the cover is opened, it can be sealed and stored in 2~8℃ away from light within 24 hours. After the cover is opened, it should be sealed and stored in -20±5℃ away from light for a long time. Transport with temperature below 0℃.

[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 , and the tissue should be fixed by conventional dehydration and paraffin embedding.

[Instructions]

1. Pre-hybridization or Pretreatment
Baking: Slides heating at 80℃ for 30min or 65℃ for 2h or overnight.
Dewaxing: According to the customer laboratory protocol (Commonly with Xylene for 15min).

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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 100℃ 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 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℃ for 5 min (the hybridizer should be preheated to 85℃) and hybridized at 42℃ 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 slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC (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) solution and wash for 2min.
④ Take out the sample and immerse it in deionized water preheated at 37℃ 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 (10x) confirm the cell area under the microscope; Go to 40x under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100x) the FISH results of nuclei were observed.

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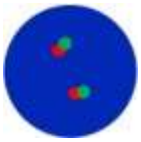
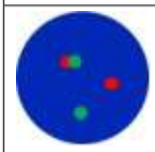
[Common Signal Type Interpretation]

FOXO1 gene site 5' signal FOXO1gene site 3' signal	
	Negative：2 fusion
	Positive : 1 orange 1 green 1 fusion

[Precautions]

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.

③ All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed off.