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Product Catalogue Number FP-130 **For clinical diagnosis and scientific research.**

TERT (5p15) Gene Break Apart Probe Detection Kit

[Product Name] TERT (5p15) Gene Break Apart Probe Detection Kit (Fluorescence In Situ Hybridization Method).

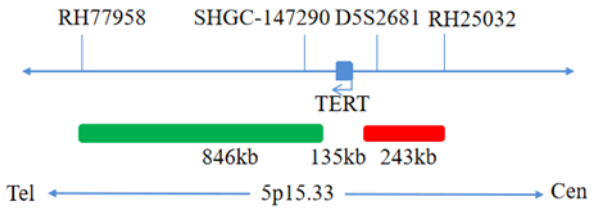
[Product Intended Use]
The reagent carries out in situ hybridization staining on the basis of routine staining to provide doctors with auxiliary information for diagnosis. The test results are only for clinical reference and should not be used as the only basis for clinical diagnosis. Clinicians should comprehensively judge the test results in combination with the patient's condition, drug indications, treatment response and other laboratory test indicators.

[Detection Principle]
Fluorescence in situ hybridization is a technique for directly observing specific nucleic acids in cells in vitro. According to the principle of base complementary pairing, the specific probe is complementary to the target sequence in the cell. Due to the fluorescence of the probe, the gene state of the hybrid probe and the target sequence can be clearly observed under the fluorescence microscope under the appropriate excitation light.

[Product Composition]
The kit consists of TERT dual-color probe as shown in Table 1.

Table 1: Kit composition

Component name	Cat.#	Specifications	Quantity	Main components
TERT dual color probe	FP-188	100μL/Tube	1	TERT Orange probe ; TERT Green probe



[Storage conditions & Validity]
This kit is shipped below 0°C. 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℃ in dark. For long-term preservation after opening, keep the lid sealed at -20°C± 5°C away from light.

[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.

[Testing Method]

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

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°C 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 should be performed in a darkroom.

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

4. Washing

The following operations should be performed in a 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°C in advance for 1min; dry it naturally in the dark place.

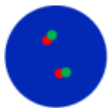
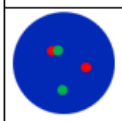
5. Counterstaining

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.

6. FISH results observation

Place the stained slides under a fluorescence microscope and confirm the cells area under a low magnification objective (10×). Under magnification objective (40×) a uniform cells distribution is observed. Then the nuclei FISH results are observed under the high magnification objective (100x).

[Common Signal Type Interpretation]

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

TERT: Orange-red (R) pattern; **TERT:** Green (G) pattern

[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. Please read this manual carefully before testing. The testing personnel shall receive professional technical training. The signal counting personnel must be able to observe and distinguish orange red and green signals.
2. When testing clinical samples, if it is difficult to count the hybridization signals and the samples are not enough to repeat the retest, the test will not provide any test results. If the amount of cells is insufficient for analysis, again, the test will not provide test results.
3. The formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity, so they need to be operated in the fume hood and wear masks and gloves to avoid direct contact.
4. The results of this kit will be affected by various factors of the sample itself, but also limited by enzyme digestion time, hybridization temperature and time, operating environment and limitations of current molecular biology technology, which may lead to wrong results. The user must understand the potential errors and accuracy limitations that may exist in the detection process.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical wastes and should be properly disposed of.
6. This product is for clinical diagnosis and scientific research.

[Manuscript version and approval date]

Manual version: V1.2 reviewed on 07 December 2021.
Manual version: V1.0 approval date 10 April 2020.