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Product Cat. No.: GBS-010

For Research Use Only.

ERG, TMPRSS2, ETV1, ETV4 gene anomaly probe detection kit Instructions Manual

[Product Name] ERG, TMPRSS, ETV1, ETV4 gene anomaly probe detection kit.

[Package Specifications] 10 Tests/box.

[Intended Usage]

This kit uses fluorescence in situ hybridization to detect the gene status of ERG, TMPRSS2, ETV1, and ETV4. The test samples are paraffin embedded tissue samples from suspected or diagnosed prostate cancer patients.

Prostate cancer is a common malignant tumor in men. In recent years, the incidence of prostate cancer in China has shown an obvious upward trend. About 80% of prostate cancer patients are accompanied by genetic changes of the fusion of TMPRSS2 gene and ETS gene family (such as ERG, ETV1, ETV4), but these changes do not occur in benign prostate diseases. FISH testing can provide more accurate and objective diagnostic indicators for clinical use compared to PSA screening and biopsy.

This kit has only been validated for the detection performance of ERG, TMPRSS2, ETV1, and ETV4 genes. This kit is only suitable for detecting the gene status of ERG, TMPRSS2, ETV1, and ETV4, providing diagnostic assistance for physicians.

[Detection Principle]

Fluorescence in situ hybridization is a technique that directly observes specific nucleic acids in cells. According to the principle of complementary base pairing, a specific DNA sequence complements and binds to the target sequence within the cell. Due to the fluorescence of the probe, under appropriate excitation light irradiation, the hybrid probe and target DNA can be clearly observed under a fluorescence microscope.

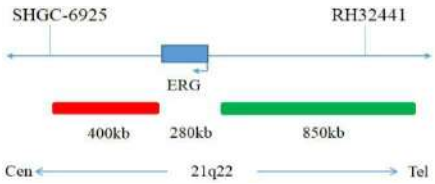
This kit uses orange fluorescent labeled orange red probes (ERG gene 5 'end), TMPRSS2 probes, and green fluorescent labeled green probes (ERG gene 3' end), ETV1, and ETV4 probes to bind the two probes to the target detection site through in situ hybridization technology. When ERG gene recombination occurs, the ERG dual color probe signal separation appears as a distant monochromatic signal. When TMPRSS2 gene and ETV1 gene fuse, the TMPRSS2/ETV1 dual color probe signal fuses to form a yellow fusion signal. When TMPRSS2 gene and ETV4 gene fuse, the TMPRSS2/ETV4 dual color probe signal fuses to form a yellow fusion signal.

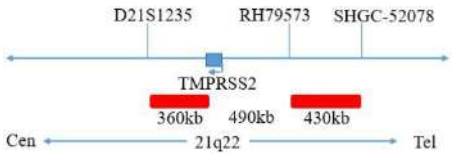
[Product Composition]

The kit consists of one of TMPRSS2/ETV1, TMPRSS2/ETV4, ERG dual color probes, as shown in Table 1.

Table 1: Kit composition

Cat#	Component name	Specifications	Quantity	Main components
FP-010-1	ERG dual color probe	100μL/Tube	1	ERG Orange probe; ERG Green probe
FP-010-2	TMPRSS2/ETV1 dual color probe	100μL/Tube	1	TMPRSS2 Orange probe; ETV1 Green probe
FP-010-3	TMPRSS2/ETV4 dual color probe	100μL/Tube	1	TMPRSS2 Orange probe; ETV4 Green probe





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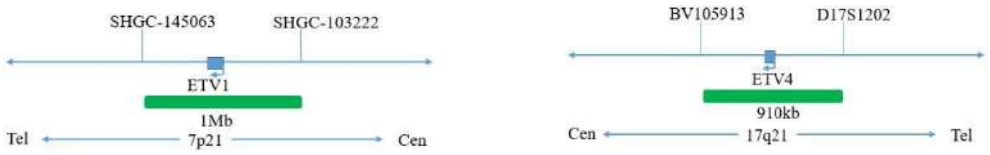

[Storage conditions & Validity]

Keep sealed away from light at -20°C±5°C. The product is valid for 20 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 is transported below 0°C.

[Applicable Instruments]

Fluorescence microscopy imaging systems including fluorescence microscopy and filter sets suitable for DAPI, Green, and Orange.

[Sample Requirements]

1. Applicable specimen type: Paraffin embedded tissue samples from suspected or diagnosed prostate cancer patients.
2. The specimen should be fixed with 4% neutral formalin fixation solution within 1 hour after being detached, and the specimen should be regularly dehydrated and paraffin embedded after fixation.
3. The thickness of paraffin slices will affect the experimental results, with a slice thickness of 4-5μm is appropriate.
4. Representative tumor tissue wax blocks should be selected from paraffin embedded tissue specimens and confirmed by HE staining.
5. It is recommended to choose paraffin embedded tissue specimens with a shorter storage time (within 5 years).

[Test Method]

1 . Related reagents

The following reagents are required for the experiment but are not provided in this kit.

① 20×SSC, pH 5.3±0.2

Sodium chloride	176g
Sodium citrate	88g

Weigh 176g of sodium chloride and 88g of sodium citrate, dissolve in 800mL of deionized water, adjust the pH to 5.3±0.2 at room temperature, and complete to 1 L with deionized water. High-pressure steam sterilization, stored at 2~8°C, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

② 2×SSC, pH 7.0±0.2

Take 100mL of the above 20xSSC, dilute with 800mL deionized water, mix, adjust the pH to 7.0±0.2 at room temperature, complete to 1L with deionized water, stored at 2~8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

③ Ethanol Solution: 70% ethanol, 85% ethanol

Dilute 700ml, 850ml of ethanol with deionized water to 1L. The shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

④ Pepsin

Protease diluent: Take 1000mL of distilled water and pour it into a 1000mL blue cap jar. Add 1.9mL of hydrochloric acid (concentration 36-8%), mix upside down, pH value about 2.0, store at 2-8 °C, shelf life of 6 months.

Protease working solution (0.5%): Dissolve 0.5g of pepsin in 100mL of protease diluent, mix well, and prepare for use.

⑤ 0.3% NP-40/0.4xSSC solution, pH 7.0-7.5

NP-40	0.6mL
20xSSC	4mL

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Take 0.6mL NP-40 and 4mL 20×SSC, add 150mL deionized water, mix, adjust the pH to 7.0-7.5 at room temperature, with deionized water complete to a volume of 200mL. Stored at 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.
⑥ Diamidinylphenyl indole (DAPI) staining agent, please choose a commercially available DAPI staining agent containing an anti-quenching agent.
⑦ Xylene.

2. Pretreatment before hybridization

It is recommended to choose known positive and negative specimens as external controls.
① Slicing: Tissue slices fixed with neutral formalin and embedded in paraffin are placed on clean slices.
② Baking: Place tissue slices on a baking machine at 65 ℃ overnight to bake and age the slices.
③ Dewaxing: Soak tissue slices in a dye vat containing xylene for 10 minutes, repeat the process, and then immediately immerse in 100% ethanol for washing for 5 minutes.
④ Rehydration: At room temperature, place tissue slices in 100% ethanol, 85% ethanol, and 70% ethanol for 2 minutes each, then immerse them in deionized water for 3 minutes. After removing the slices, use a lint free tissue to absorb excess water around the tissue.
⑤ Water treatment: Soak tissue slices in deionized water for 30-40 minutes in a 95 ℃ water bath (deionized water should be preheated in a water bath).
⑥ Washing: Soak tissue slices in 2 at room temperature × Rinse twice in SSC solution for 5 minutes each time.
⑦ Protease treatment: Immerse tissue slices in preheated 37 ℃ protease working solution for 20-40 minutes (the protease working solution is ready for use and discarded after one use).
⑧ Washing: Soak tissue slices in 2 at room temperature × Rinse twice in SSC solution for 5 minutes each time.
⑨ Dehydration: Place the tissue slices in 70% ethanol, 85% ethanol, and 100% ethanol for 2 minutes each, and then remove them and dry them naturally.

3 . Denaturation and Hybridization

The following operations need to be carried out in a dark room.
① Take out three pre prepared glass slides and mark them. Take out three sets of dual color probes, TMPRSS2/ETV1, TMPRSS2/ETV4, and ERG, and let them stand at room temperature for 5 minutes. Gently tap the bottom of the centrifuge tube with your fingers, mix the probes well, and centrifuge briefly. Take 10μL Drop onto the tissue hybridization area and immediately cover with 22mm× 22mm cover glass, the probe should be evenly unfolded without bubbles under the cover glass and sealed with rubber tape (the sealing must be thorough to prevent the dry film from affecting the detection results during the hybridization process).
② Place the slices on a hybridizer, co denature at 85℃ for 5 minutes (hybridizer should be preheated to 85℃ in advance), and hybridize at 42℃ for 2-16 hours.

4. 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×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.

5. Counterstaining

The following operations should be performed in a darkroom.
Dip 10-15μL of DAPI counterstain into the hybridization area of the glass slide, immediately cover, and then use the suitable filter to observe the sections under the fluorescence microscope.

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6. 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.

[Positive value determination or Reference interval]

1 Signal classification and counting

① Normal signal (without fusion): The ERG dual color probe in the cell displays two yellow signals or two adjacent red green signals, the TMPRSS2/ETV1 dual color probe in the cell displays two orange red signals and two green signals, and the TMPRSS2/ETV4 dual color probe in the cell displays two orange red signals and two green signals.

② Abnormal signal (fusion): When the ERG gene is fused, there are separated orange red and green signals in the ERG dual color probe in the cell. When TMPRSS2/ETV1 is fused, there is a yellow signal or a red green signal adjacent to TMPRSS2/ETV1 in the cell. When TMPRSS2/ETV4 is fused, there is a yellow signal or a red green signal adjacent to TMPRSS2/ETV1 in the cell.

2 FISH result judgment

To determine the anomalies in the detection results, it is necessary to establish an anomaly threshold

① ***Exception threshold establishment***

1) It is recommended to select pathological specimens from 20 prostate cancer patients as negative controls.

2) Use the above methods and steps to prepare glass slides for FISH experiments.

3) Establishment of abnormal threshold: 100 cells were analyzed for each probe group in each sample, and the percentage of different types of abnormal signals in each probe group was counted. The average and standard deviation of the percentage of cells displaying abnormal signals were calculated. The abnormal threshold was defined as the average+3 × Standard deviation.

Abnormal threshold=mean (M)+3 × Standard Deviation (SD).

② ***Result judgment:***

If the detection value of cell count in abnormal signal mode is greater than the abnormal threshold, it is judged as a positive result; If the detection value of cell count in abnormal signal mode is less than the abnormal threshold, it is judged as a negative result; If the detection value of cell count in abnormal signal mode is equal to the abnormal threshold, increase the observation sample cell count to 200 cells to determine the final result.

[Test method limits]

This kit is used for paraffin embedded specimens of prostate cancer tissue and is not recommended for use in other tissues. The detection ability of paraffin tissue samples that have been stored for too long cannot be evaluated according to this instruction; Follow the procedures provided in this manual, as changing the procedures may alter the results of the inspection; This kit only detects the gene status of ERG, TMPRSS2, ETV1, and ETV4, and cannot be used as the sole basis for diagnosis, prognosis judgment, or other clinical management of prostate cancer patients. A comprehensive evaluation should be conducted based on the patient's medical history and other diagnostic results.

[Product performance index]

1 Fluorescence signal intensity: After effective hybridization with the karyotype reference material, the probe should emit a fluorescence signal that can be recognized by the naked eye under a fluorescence microscope.

2. Effective rate: Three negative reference samples and three positive reference samples were tested, and the accuracy rate of the results was 100%.

3 Sensitivity:

3.1 Sensitivity of TMPRSS2 gene probe

After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 21 from 50 cells in the metaphase division phase, and at least 98 chromosomes 21 showed an orange red fluorescence signal.

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3.2 ETV1 gene probe sensitivity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 7 from 50 cells in the metaphase division phase, and at least 98 chromosomes 7 showed 1 green fluorescence signal.
3.3 ETV4 gene probe sensitivity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 17 from 50 cells in the metaphase division phase, and at least 98 chromosomes 17 showed 1 green fluorescence signal.
3.4ERG Orange Red Probe Sensitivity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 21 from 50 cells in the metaphase division phase, and at least 98 chromosomes 21 showed an orange red fluorescence signal.
3.5 ERG Green Probe Sensitivity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 21 from 50 cells in the metaphase division phase, and at least 98 chromosomes 21 showed 1 green fluorescence signal.
4. Specificity:
4.1 TMPRSS2 gene probe specificity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 21 from 50 cells in the metaphase division phase, and at least 98 chromosomes 21 displayed a specific orange red fluorescence signal in the long arm target area.
4.2 ETV1 gene probe specificity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 7 from 50 cells in the metaphase division phase, and at least 98 chromosomes 7 displayed a specific green fluorescence signal in the short arm target region.
4.3 ETV4 gene probe specificity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 17 from 50 cells in the metaphase division phase, and at least 98 chromosomes 17 displayed a specific green fluorescence signal in the long arm target area.
4.4 ERG orange probe specificity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 21 from 50 cells in the metaphase division phase, and at least 98 chromosomes 21 displayed a specific orange red fluorescence signal in the long arm target area.
4.5 ERG green probe specificity
After effective hybridization with karyotype reference materials, the probe analyzed 100 chromosomes 21 from 50 cells in the metaphase division phase, and at least 98 chromosomes 21 displayed a specific green fluorescence signal in the target region.

[Precautions]

1. Please read this manual carefully before testing. Testing personnel should receive professional technical training, and signal counting personnel must be able to observe and distinguish orange red and green signals.
2.When testing clinical samples, when the hybridization signal count is difficult and the sample is not sufficient to repeat the retest, the test will not provide any test results. If the cell count is insufficient for analysis, the test will also not provide test results.
3 .The DAPI dye used in this experiment has potential toxicity or carcinogenicity, and should be operated in a fume hood, wearing masks and gloves to avoid direct contact.
4 . The results of this reagent kit may be affected by various factors within the sample itself, as well as limitations such as enzyme digestion time, hybridization temperature and time, operating environment, and limitations of current molecular biology technology, which may lead to incorrect interpretation results. Users must understand the potential errors and limitations of accuracy that may exist during the testing process.
5. All chemicals have potential hazards and should be avoided from direct contact. Used reagent kits are clinical waste and should be properly disposed of.