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Product Cat. No.: FP-105

For Clinical Diagnosis & Scientific Research. CE

3p probe reagent
Instructions Manual

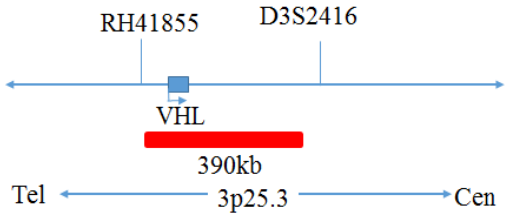
[Product Name] 3p probe reagent.
[Package Specifications] 10 Tests/box.

[Product Introduction]
This kit uses Orange fluorescein labeled VHL probe and Green fluorescein labeled CEP3, to combine VHL/CEP3 genes with the target site by in situ hybridization.

[Product Main Components]
The kit consists of VHL/CEP3 dual color probe as shown in Table 1.

Table 1: Kit composition

Component name	Specifications	Quantity	Main components
VHL/CEP3 dual color probe	100μL/Tube	1	VHL Orange probe ; CEP3 Green probe



[Storage conditions & Validity]
The kit is transported 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°C 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. Sample 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 100oC 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.

3. 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 place the slides into 2xSSC for 5 seconds, and gently remove the coverslip.
② Place the glass slides in 2xSSC 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 (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 Immerse the glass slides in deionized water at 37°C for 1min, and dry naturally in the dark.

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 are observed.

[Common Signal Type Interpretation]

VHL signal CEP3 signal	
	**Negative**: 2 Orange & 2 Green (Negative: 2R-2G)
	**Positive**: 1 Orange ; 2 Green [Positive: 1R-2G]

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

[Basic information]

Name of registrant / Manufacturer: Wuhan HealthCare Biotechnology Co., Ltd.

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[Manual Approval date & Revision date]

V1. 0: Approval date: August 7, 2020.

V1. 2: Revision date: December 7, 2021.