Wuhan HealthCare Biotechnology Co., Ltd.
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For Professional Use

PD-L1(9p24)/CSP9 gene amplification probe reagent Instructions Manual

[Product Name] PD-L1(9p24)/CSP9 gene amplification probe reagent

[Package Specifications] 10 Tests/box

[Product Introduction]

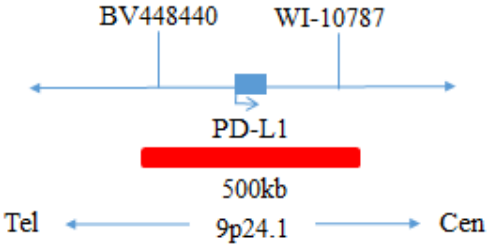
This kit uses orange fluorescein and green fluorescein to label PD-L1(9p24)/CSP9 probes. PD-L1(9p24)/CSP9 dual color probe can be combined with the target detection site by in situ hybridization.

[Product Content]

This kit consists of PD-L1(9p24)/CSP9 dual color probe, as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
PD-L1(9p24)/CSP9 dual color probe	100μl/Tube	1	PD-L1 orange probe, CEP9 green probe, hybridization buffer



[Storage conditions & Validity]

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. The kit is transported under 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.

[Test method]

It is recommended to use Wuhan HealthCare Biotechnology Co., Ltd.'s "FISH Pretreatment Reagent " (Cat# CL-003) for pretreatment.

2. Denaturation and Hybridization

The following operations need to be carried out in the darkroom.

① Take out the probe, let it stand at room temperature for 5min, turn it upside down with force, fully mix the probe, and then centrifuge briefly (vortex instrument oscillation is prohibited), take 10μL was dropped on the hybridization area of cell drops and immediately covered with 22mm×22mm cover glass, the probe shall be evenly expanded under the cover glass without bubbles, and the edge shall be sealed with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results during hybridization).

② Put the tissue sections on the hybridizer, CO denature at 85°C for 5min (the hybridizer should be preheated to 85°C in advance), and hybridize at 42°C for 2-16h.

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 sample and immerse it in 0.3%NP-40/0.4xSSC solution preheated at 68°C 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 environment.

4. Counterstaining

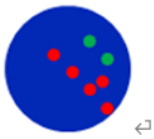
The following operations should be performed in a darkroom

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 (10 ×) Confirm the cell area under the microscope; Go to 40× Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100 ×) The FISH results of nuclei were observed.

[Common Signal Type Interpretation]

PD-L1 gene site signal CEP9 gene site signal	
	Negative :2 Orange, 2 Green
	Positive: n Orange 2 Green (n≥3)

[Precautions]

① This product cannot be used for clinical diagnosis, but only for scientific research.

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. Used kits are hazardous wastes and should be properly disposed of.

[Basic information]

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

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[medical device registration certificate No. / product technical requirement No.]

Medical device registration certificate No.: ehxb20190903

[approval date and modification date of the specification]

V1. 0 approval date: October 24, 2019

V1. 2 approval date: December 7, 2021