

# Immunohistochemistry kit SL-IHC100T

Period of validity:One year. Specific: 100T

# Reagents supplied:

	Product	Size	Storage	Remark
1	Citrate buffer (0.01mol/L, pH6.0)	1L	4℃	Solute in 1L of deionized water or distilled water
2	PBS buffer	2L	4℃	Solute in 2L of deionized water or distilled water
3	DON-HRP-Conjugated polyase (anti mouse / rabbit)	6mL	4℃	Non-frozen, 50μL per slice
4	30% H2O2	10mL	4℃	Dilute into 3%, ready to use
5	DAB solution I	0.6mL	-20℃	Ready to use
6	DAB solution II	0.6mL	-20℃	Ready to use
7	hematoxylin	6ml	4°C	-

Please store the kit separately according to the storage temperature requirements, and do not use the expired kit.

# The other relate Reagents(Materials be required but not be supplied):

1	absolute ethyl alcohol	500mL
2	95% alcohol	500mL
3	dimethylbenzene	500mL
4	Neutral gum	100mL/bottle
5	4% paraformaldehyde	100mL/bottle
6	HE Staining kit	3×10mL
7	Primary antibody dilution	10mL
8	Closed solution (normal goat serum)	10mL
9	5% BSA sealing fluid	10mL
10	Sectioning box	12/25/50/100
11	0. 1% trypsin liquid digestive solution	10mL
12	Primary antibody(anti mouse/Rabbit)	10/20/50/100ul

The above items need to be purchased separately or buy it from sunlong biotech.



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# **Staining procedure:**

# 1. Grilling Slices and dewaxing

The slides bake in a 65°C constant-temperature oven for 1h; immerse in xylene I for 15min, and then immerse in xylene II for 15min.

#### 2. Hydration

The dewaxed sections soak with 100% alcohol I, 100% alcohol II, 95% alcohol, 85% alcohol and 75% alcohol, and rinsed for 10min with tap water.

#### 3. Antigen Repair

Repair 15min in medium with 0.01M sodium citrate buffer solution (reference 125  $^{\circ}$ C • 103KPa) for natural cooling

#### 4. blocking-up

Put the slides at 3% H2O2, incubate in wet boxe for 10min to eliminate the endogenous peroxidase activity. Wash it by PBS solution for 3min\*3 times.

#### 5. seal off

Put the slides in 5% BSA, incubate in wet boxes for 30min, and wash it by PBS solution for 3min\*3 times.

## 6. Incubation primary antibody

Add primary antibody (select optimal dilution ratio), incubate in wet box and overnight at 4 °C, wash it by PBS solution for 3min\*3 times.

#### 7. Incubation secondary antibody

Remove the PBS solution, add 50 µL of DON-HRP-Conjugated, incubate in wet box, and place it for 20min-30min at room temperature. Wash it by PBS solution for 3min\*3 times

## 8. Coloration

DAB staining (mix DAB solution I and DAB solution II at 1:1) and when the sections have color change, wash the staining solution by tap water immediately.

## 9. Hematoxylin staining

Hematoxylin re-fecte for 3min, 1% hydrochloride alcohol differentiation and microscopy to control the degree of staining. Rinse for 10min by Tap water.

#### 10. Dehydrate

Samples soak in 75%, 85%, 95%, 100% alcohol I, and 100% alcohol II for 5min.

# 11. Transparency and sealing

Slides were placed in xylene for 3min\*2 times, transparent and neutral gum sealed

#### 12. Images acquisition and analysis

Photo pictures by a microscope, collect and analyze sample-relate sites.