SunLong Biotech Co.,LTD
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Immunohistochemistry kit

SL-IHC200T

Period of validity: One year. **Specific：** 200T

Reagents supplied：

	Product	Size	Storage	Remark
1	Citrate buffer (0.01mol/L, pH6.0)(Powder)	1L	4℃	Solute in 1L of deionized water or distilled water
2	PBS buffer(Powder)	2L	4℃	Solute in 2L of deionized water or distilled water
3	Enzyme labeled secondary antibody polymer (anti mouse / rabbit)	12mL	4℃	Non-frozen, 50μL per slice
4	30% H2O2	20mL	4℃	Dilute into 3% , ready to use
5	DAB solution I	1.2mL	-20℃	Ready to use
6	DAB solution II	1.2mL	-20℃	Ready to use
7	Hematoxylin	12ml	4℃	-

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℃ 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 ℃ • 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 ℃, 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.