SUNLONG

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Rat CD31 Ready-To-Use IHC Kit

Catalog Number: SL-IHC003

Size: 50T

Assay type: Immunohistochemistry (IHC-P)

Reactivity: Rat

Sample type: FFPE tissue

Storage: 2-8℃ (see individual tube labels); stable for 6 months from date of receipt

GENERAL INFORMATION

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	1×	RT
2	Antigen Retrieval Buffer	20 mL	100×	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 mL	RTU	2-8℃, protect from light
4	Blocking Buffer	3 mL	RTU	2-8℃
5	Primary Antibody (Rat CD31 Rabbit pAb)	6 mL	RTU	2-8℃
6	Secondary Antibody (Goat Anti-Rabbit IgG H&L, HRP conjugated)	6 mL	RTU	2-8℃
7	Chromogen Component A	0.3 mL	RTU	-20℃, protect from light
8	Chromogen Component B	0.3 mL	RTU	-20℃
9	Counter Staining Reagent	5 mL	RTU	RT
10	Mounting Media	5 mL	RTU	RT
11	Control slide (Rat liver)	1 slide	RTU	RT
12	Datasheet	1 copy	-	-

IMMUNOHISTOCHEMISTRY PROTOCOL

1. Deparaffinization and rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80% and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

2. Antigen retrieval

Add 100× Antigen Retrieval Buffer into distilled water to prepare a 1× solution. Boil slides in the 1× solution at 95-100℃ for 15 minutes. Move the slides to 1× solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with PBS Buffer (dissolve the powder in 2 L distilled water) for 5 minutes each.

3. Block endogenous peroxidase

Drain the liquid off the slides and use a hydrophobic IHC pen to draw circles around the tissue sections. Add 2-4 drops of Endogenous Peroxidase Blocking Buffer directly on the slides, covering the whole tissue, and block for 15 minutes at RT. Rinse 3 times with PBS Buffer for 5 minutes each.

4. Serum blocking

Block with 2-4 drops of Blocking Buffer for 20 minutes at RT.

5. Primary antibody incubation

Drain the blocking buffer from the slides. Incubate the slides with 2-4 drops of Rat CD31 Rabbit pAb overnight at 4℃ or for 1-2 hours at RT. Rinse 3 times with PBS Buffer for 5 minutes each.

6. Secondary antibody incubation

Incubate the slides with 2-4 drops of Goat Anti-Rabbit IgG H&L, HRP conjugated for 1-2 hours at RT. Rinse slides 3 times with PBS Buffer for 5 minutes each.

7. Signal development

Remove residual liquid around the tissue section. Add 50 μL fresh DAB Buffer (Chromogen Component A : Chromogen Component B : PBS Buffer = 1 : 1 : 18) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximately 3-5 minutes at RT). Stop the reaction immediately by rinsing with distilled water; rinse slides 3 times with distilled water for 5 minutes each.

8. Counterstain

Counterstain with an appropriate amount of Counter Staining Reagent for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of Differentiation reagent to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

9. Dehydration and mounting

Immerse slides sequentially in 70%, 80%, 90%, 95% and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some Mounting Media on the tissue and mount coverslips.

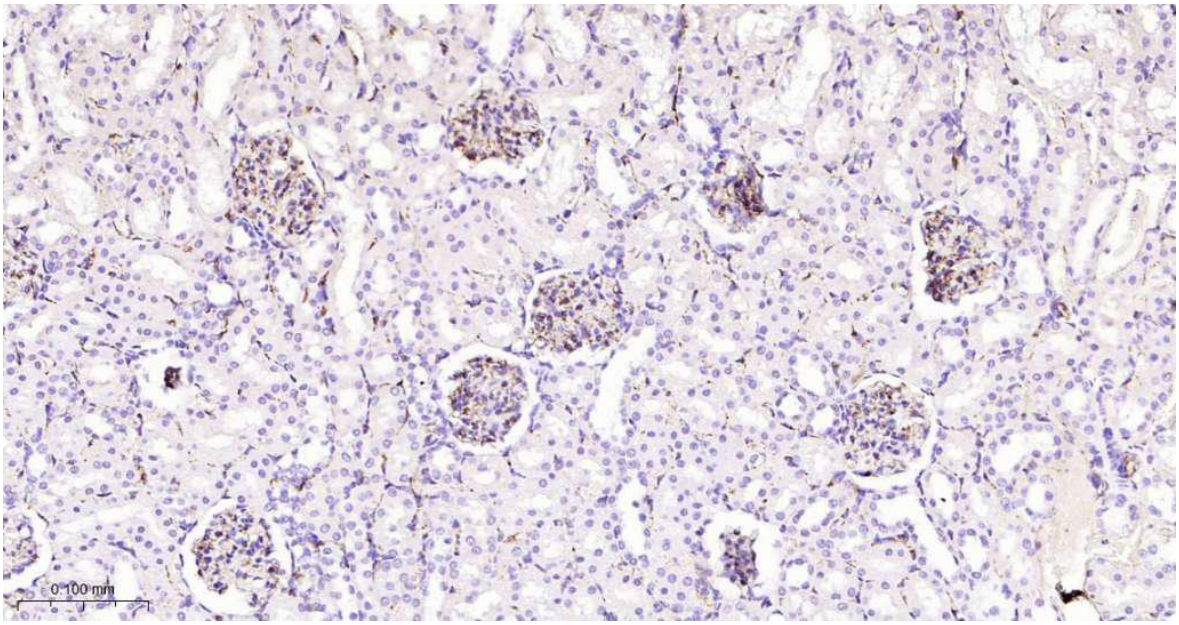
NOTES

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please do not replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4℃ after preparation; the antigen retrieval buffer (1× reagent 2) and the chromogenic agent (mixture of reagents 7 and 8) should be prepared right before each assay.

INTRODUCTION

CD31 (platelet endothelial cell adhesion molecule-1, PECAM-1) is an inhibitory coreceptor involved in regulation of T cell and B cell signaling by a dual immunoreceptor tyrosine-based inhibitory motif (ITIM) that, upon associated kinase-mediated phosphorylation, provides docking sites for protein-tyrosine phosphatases. CD31 is expressed ubiquitously within the vascular compartment and is located mainly at junctions between adjacent cells. The N-terminal Ig-like domain of CD31 is responsible for its homophilic binding, which plays an important role in cell-cell interactions. CD31 is a multifunctional molecule with diverse roles in modulation of integrin-mediated cell adhesion, transendothelial migration, angiogenesis, apoptosis, negative regulation of immunoreceptor signaling, autoimmunity, macrophage phagocytosis, IgE-mediated anaphylaxis and thrombosis. It is one of the key regulatory molecules in the vascular system.

VALIDATION DATA



Immunohistochemical analysis of paraffin-embedded Rat kidney tissue slide using this kit (Cat. No. SL-IHC003).

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