HistoSure
A SySy brand

Excellent Antibodies for Histopathology

Reference Protocol for Anti-CD4 (HS-360 004) Immunohistochemistry using DAB as Chromogen

Tissue Fixation

- 3.7% formaldehyde (24 h), 3.5 µM paraffin sections

Materials and Reagents

• Food Steamer	Braun, Multigourmet	
• Staining Containers with slide holders (e.g. Tissue-Tek)		
• Protein Block, Serum-Free	Agilent	X0909
• Antibody diluent	Agilent	S2022
• Biotinylated anti-guinea pig antibody	Jackson	106-065-003
• ABC HRP Kit, Standard	Vectorlabs	PK-4000
• ImmPACT DAB	Vectorlabs	SK-4105
• Hydrogen peroxide 30%	Merck	1.07298.0250
• PBS (pH 7.4)		
• TBST (TBS, 0.05% Tween 20, pH 7.6)		
• Antigen Retrieval buffer: **Citrate Buffer (10 mM Citrate, 0.05% Tween 20, pH 6.0)**		
• Xylene, 100% ethanol, 90% ethanol, 80% ethanol and 70% ethanol, 2-propanol		
• Optional: Hematoxylin Solution (Mayer's, Modified) or other nuclear counterstain		
• Optional: Avidin/Biotin Blocking Kit	Vectorlabs SP-2001	
• Non-aqueous mounting medium		

Method

1) **Deparaffinize and hydrate tissue sections**
   a) Xylol 2 x 5 min
   b) 100% EtOH 2 x 2 min
   c) 90% EtOH 1 x 2 min
   d) 80% EtOH 1 x 2 min
   e) 70% EtOH 2 x 2 min
   f) Deionized Water 1 x 20 sec
   g) PBS 1 x 2 min

*Keep the slides in PBS until ready to perform the Antigen Retrieval.
Do not allow the slides to dry out*

2) **Antigen Retrieval (AR) using a food steamer**
   a) Heat the steamer with a suitable staining container filled with Antigen Retrieval buffer to **~97°C**
   b) Transfer the sections into the staining box, wait until the temperature reaches **97°C**
   c) Incubate the sections in the steamer for **30 min**
   d) Remove the staining container from the steamer and allow the slides to cool down for **20 min** (target end temperature **~60°C**)

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3) Wash slides in PBS, 3 x 1 min

4) **Blocking endogenous peroxidase activity**
   a) Incubate the sections with 3% hydrogen peroxide in PBS (freshly prepared!) for **5 min**

5) Wash slides in PBS, 2 x 1 min
6) Wash slides in TBST, 1 x 2 min

7) **Optional:** Perform Avidin-Biotin-Block according to manufacturer's instructions.
   Note: Certain tissues (e.g. liver, kidney) contain high levels of endogenous biotin. The Avidin-Biotin blocking step is recommended when using the ABC system for these tissues. If the background problem persists, consider trying a polymer-based detection system instead of biotinylated secondary antibody / ABC system.

8) Block in Protein Block, Serum-Free for **10 min**
9) **Drain slides (do not rinse)**
10) **Apply primary antibody diluted in Antibody Diluent** and incubate in a humidified chamber for **1 h at room temperature**
    Suggested dilution: 1:500 – 1:1000 in Antibody Diluent

11) Wash slides in TBST, 3 x 2 min

12) **Apply secondary antibody diluted in Antibody Diluent for 30 min at room temperature.**
    ***Suggested concentration:** 5 µg/ml*
    Perform step 13 in the interim

13) **Prepare the ABC-reagent:** 5 ml PBS + 1 drop A + 1 drop B, incubate for 30 min
14) Wash slides in TBST, 3 x 2 min
15) **Apply the ABC reagent** for **30 min at room temperature**
16) Wash slides in TBST, 3 x 2 min
17) **Apply the DAB substrate**, **1-10 min**

    *Observe the staining with a microscope!
    Development times may differ depending upon the level of antigen*

18) Stop the DAB reaction with deionized water
19) **Optional: Counterstain**
    a) Follow the manufacturer's instructions for counterstaining and bluing

20) Wash slides in deionized water for 1 min

21) **Dehydrate tissue sections:**

a) **70% EtOH**	**2 x 10 sec**
b) **80% EtOH**	**1 x 10 sec**
c) **90% EtOH**	**1 x 10 sec**
d) **2-Propanol**	**2 x 1 min**
e) **Xylol**	**3 x 2 min**

22) **Mount slides in a suitable organic mounting medium and add coverslip**

Note: The SYSY standard protocol generates good results in the SYSY labs and may be used as a reference. However, to achieve the highest specific signal and lowest non-specific background signal, the best antigen retrieval condition, antibody concentration, incubation temperature, and incubation time must be determined individually. Please also refer to our general protocols.

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