

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)
- 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 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
 b. 80% EtOH
 c. 90% EtOH
 d. 2-Propanol
 e. Xylol
 2 x 10 sec
 1 x 10 sec
 2 x 1 min
 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.