SYSY | Histo*Sure*

Reference Protocol for Anti-CD11b (HS-384 308) 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: 1:400 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

Synaptic Systems
Rudolf-Wissell-Str. 28a
37079 Göttingen, Germany

Page 2 of 3

Web: https://sysy-histosure.com
Tech. support: support@sysy.com

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.

Synaptic Systems
Rudolf-Wissell-Str. 28a
37079 Göttingen, Germany

Page 3 of 3

Web:https://sysy-histosure.com
Tech. support: support@sysy.com