SYSY | HistoSure

# Reference Protocol for Anti-CD86 (HS-466 003) 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-rabbit antibody | Jackson | 111-065-144 |
| • 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:<br>**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*

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   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*

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