SYSY | HistoSure

# Reference Protocol for CD11c (HS-375 008) Immunohistochemistry using Fluorescence

## 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 |
| • Fluorophore conjugated secondary antibody e.g., Donkey anti-rabbit Cy3 | Jackson | 711-165-152 |
| • PBS (pH 7.4) | | |
| • 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: DAPI (4 mg/ml in dH2O) | Roth | 6335.1 |
| • Mounting medium: glycerol-based, hard-setting, ready-to-use mountant | | |

## 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. Block in Protein Block, Serum-Free for **10 min**
5. **Drain slides (do not rinse)**
6. **Apply primary antibody diluted in Antibody Diluent** and incubate in a humidified chamber **over-night at 4°C**
   ***Suggested dilution: 1:400 in Antibody Diluent***
7. Wash slides in PBS, 3 x 2 min
8. **Apply secondary antibody diluted in PBS for 60 min at room temperature.**

***Suggested concentration:** 2.5 µg/ml*

***Note:*** *Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye.*

9. Wash slides in PBS, 3 x 2 min
10. **Optional: Counterstain**
    a. Dilute DAPI stock solution 1:10,000 in PBS and apply for 5 min.
    b. Wash slides in PBS, 3 x 1 min
11. **Mount slides 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. High levels of autofluorescence in tissue samples (e.g., mouse spleen) can impair immunofluorescence histochemistry. Using an Autofluorescence Quenching Kit can diminish unwanted autofluorescence and improve signal-to-noise ratio. Please also refer to our general protocols.*