SYSY | HistoSure

Reference Protocol for MEF2A (HS-507 003) 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: **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: 1:4000 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.*