

# Reference Protocol for biotinylated Anti-IBA1 (HS-234 017BT) 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
 Agilent
 S2022
 ABC HRP Kit, Standard
 ImmPACT DAB
 Yectorlabs
 Yectorlabs
 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.	<b>Deionized Water</b>	1 x 20 sec
g.	PBS	1 x 2 min

<sup>\*</sup>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: 1:100 in Antibody Diluent\*
  \*Perform step 11 in the interim\*
- 11. Prepare the ABC-reagent: 5 ml PBS + 1 drop A + 1 drop B, incubate for 30 min
- 12. Wash slides in TBST, 3 x 2 min
- 13. Apply the ABC reagent for 30 min at room temperature
- 14. Wash slides in TBST, 3 x 2 min
- 15. Apply the DAB substrate, 1-10 min

\*Observe the staining with a microscope!

Development times may differ depending upon the level of antigen\*

- 16. Stop the DAB reaction with deionized water
- 17. Optional: Counterstain
  - a. Follow the manufacturer's instructions for counterstaining and bluing
- 18. Wash slides in deionized water for 1 min
- 19. Dehydrate tissue sections:

a. 70% EtOH 2x10 sec
 b. 80% EtOH 1x10 sec
 c. 90% EtOH 1x10 sec
 d. 2-Propanol 2x1 min
 e. Xylol 3x2 min

20. 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.