

# Code No. 012-28521 (20 $\mu L)$

018-28523 (100 µL)

# Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant

Code No.	Product name	Pkg. size	Storage
012-28521	Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant	20 µL	-20°C
018-28523		100 µL	

# Product Summary

Reactivity	Mouse, rat (not studied in other species)
Clonality	Monoclonal (Clone No. 6A4)
Host	Rabbit
Conjugate	Unconjugated
Concentration	Described on the label

# **Product Details**

Source	CHO-Spica cell derived antibody
Immunogen	Synthetic peptide corresponding to the C-terminus of Iba1
Purity	Protein A affinity chromatography
Isotype	IgG
Form	Liquid
Preservative	0.05% Sodium azide
Buffer	PBS with 50% glycerol

#### **Application**

Immunohistochemistry (frozen section) 1:200 - 10,000

\* The optimal dilutions/concentrations should be determined by each laboratory.





# Recommend Protocol

#### Step 1. Tissue preparation

- 1. The animal (mouse or rat) should be fixed by perfusing with 4% paraformaldehyde-PBS under the deep anesthesia.
- After dissection the tissue, sink the tissue in 4% paraformaldehyde-PBS for post-fixation, following to 30% sucrose/ 4% paraformaldehyde-PBS, respectively.
  \*Sink the tissue until falling-down on the bottom of tube.
- 3. Mount the tissue with sectioning compound and freeze it completely.
- 4. Prepare a 20-50  $\mu$ m thick of frozen section using the cryostat and mount the slice on the pre-coated slide glass for IHC.

# Step 2. Blocking and Permeabilization

- 1. Rinse the section several times with PBS for removing the sectioning compound.
- 2. Block the section by Blocking solution (1% animal serum, 2% BSA and 0.3% TritonX-100 in PBS) for 2 hours at room temperature.

#### Step 3. Antibody staining

- 1. Incubate the section with primary antibody solution (Anti Iba1, Rabbit Monoclonal Antibody (6A4), recombinant, 1000 times dilution in Blocking solution) at 4°C over night.
- 2. Wash the section three times with 0.3% TritonX-100 in PBS for 5 min.
- 3. Incubate the section with secondary antibody solution (i.e. fluorescent dye-conjugated anti-rabbit IgG, appropriatedilution in Blocking solution) for 2 hours RT.
- 4. Wash the section three times with 0.3% TritonX-100 in PBS for 5 min.

# Optional: Double or nuclear staining

- (a) Double staining
- Prepare the different primary and secondary antibodies than those used in "Step 3" and repeat "Step 3".
  - \*You must prepare a source of primary antibody that is different from the one used in "Antibody staining".
- (b) Nuclear stain
- Prepare DNA binding dye such as DAPI (i.e. code No. 342-07431). The staining protocol follows instruction manual of each dye.





# Step 4. Mounting and Detection

- 1. Rinse the section with ddH2O quickly, and snap-off the slide.
- 2. Absorb remaining ddH2O with paper and drop anti-fade mounding medium, then place a coverslip on the section.
- 3. Observe the section image by fluorescent microscopy or confocal microscopy with appropriate filter set.

