HistoSure
A SySy brand

Excellent Antibodies for Histopathology

Reference Protocol for Anti-IBA1 (HS-234 008) Immunocytochemistry of Primary Neuronal Cell Cultures

Materials and Reagents

- **Fixation solution:** 4% formaldehyde, 4% sucrose in PBS
- **PBS**: Phosphate buffered saline, pH 7.4
- **Blocking buffer:** 10% normal serum, 0.1% Triton X-100 in PBS (normal serum from the host-species of the secondary antibodies is recommended for blocking)
- **Incubation buffer:** 5% normal serum, 0.1% Triton X-100 in PBS, (normal serum from the host-species of the secondary antibodies is recommended for blocking)
- **Secondary antibody:** anti-rabbit secondary antibody conjugated to fluorescent dye
- **Optional:** DAPI (4 mg/ml)

Procedure:

1. Wash primary cell cultures carefully with **PBS.**
2. Cover cells with **fixation solution** and incubate for 15 min at RT.
3. Wash three times for 10 min in **PBS**.
4. Incubate for 30 min with **blocking buffer**.
5. Apply the primary antibody in **incubation solution** at a 1:250 dilution and incubate for 2 h at RT.
6. Wash three times for 10 min in **PBS**.
7. Apply the secondary antibody in **incubation solution** diluted to the manufacturer's recommended concentration and incubate for 1 h at RT. **Optional**: Add DAPI at a 1:10,000 dilution to the secondary antibody solution.

***Note**: Avoid bright light when working with the secondary antibody to minimize photo bleaching of the fluorescent dye. In Multiplex stainings make sure to use secondary antibodies cross-adsorbed against the host species of the other primary antibody used in your experiment. Ideally all secondary antibodies should come from the same host species. If not, make sure that they have been cross-adsorbed against IgGs of the host-species of the other secondary antibody as well. This avoids cross-reaction between the secondary antibodies.*

8. Wash three times for 10 min in **PBS**.
9. Mount coverslips and observe under a microscope.

***Note**: The SYSY standard protocol generates good staining 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 antibody concentration, incubation temperature and incubation time for each antibody must be individually determined. Please also refer to our general protocols.*

Synaptic Systems GmbH
Rudolf-Wissell-Str. 28a
37079 Goettingen, Germany

web: www.histosure.com
Tech. support: support@sysy.com