NEUROMICS

IBA1 Polyclonal Rabbit Antibody Data Sheet

Catalog Number:	RA22133	**Host:**	Rabbit
Product Type:	Polyclonal	**Species Reactivity:**	Human, Rat, and Mouse
Immunogen Sequence:	Peptide identical to part of the C-terminal of human IBA1 coupled to KLH.	**Format:**	Supplied as an aliquot of serum plus 5mM NaN_3
Applications:	Western Blot: 1:1,000-5,000. Immunocytochemistry/Immunofluorescence: 1:2,000-5,000 Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.		
Storage:	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.		

Application Notes

Description/Data:

IBA1 is an acronyn for “ionized calcium binding adapter molecule 1”, and the protein is also known as AIF1 for “allograft inflammatory factor 1”. AIF1 was originally identified, cloned and sequenced as a protein heavily upregulated in an animal model of graft rejection (1). The AIF1 protein was localized in macrophages and neutrophils surrounding and infiltrating the graft site. Shortly afterwords the same protein was identified as IBA1 in a screen for cytokine induced genes in neurons (2). In the event the workers identified a gene product which was neither expressed in neurons nor induced by cytokines, but which had some very interesting properties, including the important observation that IBA1 was only expressed in hematopoetic cells. IBA1 and AIF1 were subsequently found to be identical, being a small globular 17kDa molecule belonging to the “EF” hand superfamily of Calcium binding proteins. As with other related molecules IBA1 probably has a role in Calcium buffering and in the responses of cells to changes in the level of cellular Calcium. IBA1 is specifically expressed in hematopoetic cells such as neutrophils, macrophages and monocytes. Since the only hematopoetic cells normally found within the central nervous system are microglia, suitable IBA1 antibodies are widely used to identify microglial cells in sections and tissues. Microglia are the immunocompetent cells of the CNS and are extremely important in responses to injury and disease. Microglial are small but very active cells which constantly send processes probing their neighborhood and which alter morphology and are induced to divide following a variety of CNS compromises.

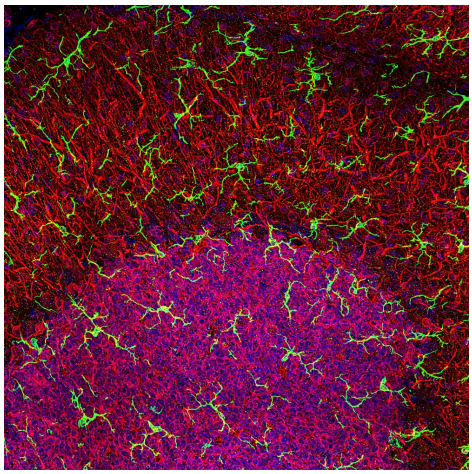

Image: High magnification stacked confocal image of rat cerebellar molecular layer at top and granular layer below, stained with RA22133, dilution 1:1,000, in green. Microglia are very small cells with fine processes spreading in three dimensions and so are best visualized in a confocal Z stack. Red shows the processes of Purkinje cells and the perikarya of granule cells revealed with, an antibody to MAP2, 1:5,000. Nuclear DNA is shown with DAPI stain in blue.

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Immunostaining of cells in tissue culture:

The purpose of fixation is denaturing the components of cells enough so that they stay on the dish and can be bound by antibodies, hopefully without destroying cellular morphology. Fixatives such as formalin, paraformaldehyde and glutaraldehyde chemically cross-link proteins, by binding to amino acid side chains, generally the most chemically reactive ones like amines (Lysine, Arginine, Glutamine and Asparagine). This chemical modification can also have the consequence of blocking antibody binding sites. Substances such as acetone and methanol are not true fixatives but are denaturants, which precipitate proteins without covalently modifying them. We routinely use a combination of mild formalin fixation followed by cold methanol for neurons, mixed neuron/glial cultures and most of the widely used human and rodent cell lines. The formalin preserves the cellular morphology quite well, while the methanol further denatures the proteins of the cells and helps keep what is left of the cell adherent to the dish. For soluble proteins it may be necessary to miss the methanol step, but in this case you have to be very careful with the washing steps, as the cells tend to wash off the dish. Certain antibodies may be very sensitive to formalin fixation, so you may have to experiment a little, perhaps missing out that step. The following procedure works for antibodies to most cytoskeletal and signaling molecules. This procedure is good for cells in 6 well tissue culture plates or in 35mm tissue culture dishes. These are just big enough that you can look from above with a typical immunofluorescence microscope through a glass coverslip. This allows you to see the specimens very well and take very high quality pictures. (However note that it's a pain to changes lenses on the microscope if you use the 6 well dishes, since you have to rack the lens right the way up to do this, and you have to take out the two neighboring lenses in the turret since they will hit the other wells of the dish! It's less of a problem with 35mm dishes but still a pain. No procedure is perfect….).

1. Draw of culture medium with aspirator and add 1 mL of 3.7 % formalin in PBS solution to the dish. (make up from 10 mLs Fisher 37% formalin plus 90mls PBS, the Fisher formalin contains 37% formaldehyde plus about 1% methanol which may be relevant sometimes). Let sit at room temp for 1 minute. (can add 0.1% Tween 20 to PBS used here and all subsequent steps to reduce background; probably best not to do this first time round though as it may extract your antigen or help wash your cells off the dish).

2. Take off the formalin/PBS and add 1ml of cold methanol (-20°C, kept in well-sealed bottle in fridge). Let sit for no more than 1 minute.

3. Take off methanol and add 1ml of PBS, not letting the specimen dry out. To block nonspecific antibody binding can add ~10 µL (=1%) of goat serum (Sigma), and can incubate for 30 minutes. Can then add antibody reagents. Typically, 100 µL of hybridoma tissue culture supernatent or 1ml of mouse ascites fluid or crude serum. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight, exact time not too critical). Can do very gentle shaking for well adherent cell lines (3T3, Hek293 etc.).

4. Remove primary antibody and replace with 1 mL of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

5. Add 0.5 µLs of secondary antibody. These are fluorescently labeled Goat anti mouse or rabbit antibodies and are conjugated to ALEXA dyes and were originally marketed by Molecular Probes (Eugene Oregon, the ALEXA dyes are sulphonated rhodamine compounds and are much more stable to UV than FITC, TRITC, Texas red etc. Molecular Probes was bought by Invitrogen, which now markets these reagents). Typically make 1:2,000 dilutions of these secondaries in PBS plus 1% goat serum, BSA or non fat milk carrier. Incubate for 1 hour at room temp. (or can go at 37°C for 30 minutes to 1 hour, or can do 4°C overnight). Can do gentle shaking for well adherent cell lines (3T3, HEK293 etc.).

6. Remove secondary antibody and replace with 1 ml of PBS. Let sit for 5-10 minutes, replace PBS and repeat twice, to give three washes in PBS.

7. Drop on one drop of Fisher mounting medium onto dish and apply 22 mm square coverslip. View in the microscope!

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