Product Data Sheet:
IBA1 Rabbit Monoclonal Antibody

Cat. No:	MAB-94785
Conjugate:	Unconjugated
Size:	100 ul
Clone:	EPR16589
Concentration:	1mg/ml
Host:	Rabbit
Isotype:	IgG
Immunogen:	Peptide identical to the C-terminal of human IBA1 coupled to KLH
Reactivity:	Hu, Ms, Rt
Applications:	Western Blot: 1:1,000-2,000. Immunofluorescence:1:1,000-2,000 Immunocytochemistry: 1:1,000-2,000 Immunohistochemistry(paraffin-tissues): 1:1000 Immunohistochemistry (frozen-tissues): 1:1000 “Free floating”
Molecular Weight:	17kDa
Purification:	Aff.Pur.
Background:	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.
Form:	Liquid
Buffer:	Supplied as an aliquot of serum plus 5mM NaN3
Storage:	Stable at 4°C for one year, for longer term store at -20°C. Avoid freeze/thaw cycles.

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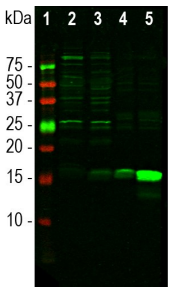
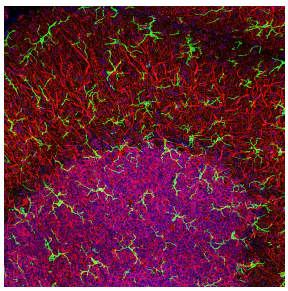
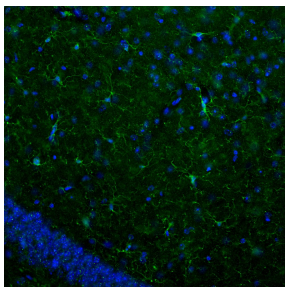


Western blot analysis of different tissue lysates using rabbit mAb to IBA1, IBA1, dilution 1:1,000 in green: [1] protein standard (red), [2] mouse brain, [3] rat brain, [4] mouse spleen, and [5] rat spleen. The band at about 15kDa mark corresponds to IBA1 protein. IBA1 is a relatively minor protein of brain and is much more abundant in spleen, so the 15kDa band is less obvious in CNS lysates. The other bands seen in the CNS lysates are of unknown origin but do not appear to compromise the migroglial specific staining seen with this antibody.



High magnification stacked confocal image of rat cerebellar molecular layer at top and granular layer below, stained with IBA1, 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.



Immunofluorescent analysis of mouse hippocampus section stained with rabbit mAb to Iba1, dilution 1:1,000 in green. The blue is Hoechst staining of nuclear DNA. Following transcardial perfusion of mouse with 4% paraformaldehyde, brain was post fixed for 24 hours, immersed to 15, then 30% sucrouse, froze and cut to 45 µM. Free-floating sections were stained with above antibodies. Microglia are very small cells with fine processes spreading in three dimensions.

PROCEDURE OF IMMUNOFLUORESCENT STAINING OF FREE-FLOATING BRAIN TISSUE SECTIONSTISSUE PREPARATION:

1. Perfuse transcardially the animal (rat or mouse) with ice-cold PBS (pH7.4), followed by freshly made 4% paraformaldehyde fixative solution in PBS.
2. Postfix the removed brain in the same 4% paraformaldehyde fixative solution in PBS (4°C for 16 – 24 hours).
3. Cryoprotect the tissue by immersing it in sucrose solutions in PBS (15%, for 24 hours followed by 30% until tissue will sink, may take from 48 hours up to 1 week).
4. Cut 40 – 50µm sections on a cryostat.
5. Keep sections in PBS + 0.05M NaN3 at 4°C

until they were taken for staining. During the staining process, the sections should never be allowed to dry out.

IMMUNOFLUORESCENT STAINING:

1. Rinse sections with PBS
2. Block and Permeabilize sections in 10% Normal Goat Serum (serum of the species the secondary antibody was made in), 1% Triton-100 in PBS for 1 hour with slight agitation.
3. Incubate sections with the primary antibody diluted in 1% Normal Goat Serum , 0.1% Triton-100 in PBS at 4°C overnight with slight agitation.
4. Rinse sections 3 times with PBS, first rinse is quick, but wait 5 minutes between each subsequent rinses. (This step is to wash away unbound primary antibody).
5. Add fluorochrome-conjugated secondary antibody, diluted 1:2,000 in 1% Normal Goat Serum , 0.1% Triton-100 in PBS, and can add Hoechst 1:2,000 (blue dye, for nuclear DNA staining). Cover plate with foil and incubate 2h at room T with slight agitation.

Note: a common example would be ALEXA Fluor anti-mouse, anti-rabbit, or anti-chicken antibodies.

6. Rinse sections 4 times with PBS, first rinse is quick, but wait 5-10 minutes between each subsequent rinses.
7. Mount sections on clean glass slides with glycerol-base mounting medium, and cover it with coverslip. Store slides at 4°C.

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