Product Data Sheet:
IBA1 Chicken Polyclonal Antibody

Cat. No:	AB-84416
Conjugate:	Unconjugated
Size:	100 ug
Clone:	POLY
Concentration:	1mg/ml
Host:	Chicken
Isotype:	IgG
Immunogen:	Peptide identical to part of the C-terminal of human IBA1 coupled to KLH
Reactivity:	Human, rat and mouse
Applications:	Western Blot: 1:500-1:2000 Immunofluorescence: 1:1,000-1: 2,500 Immunocytochemistry: 1:1,000-1: 2,500 Immunohistochemistry: 1:1,000-1: 2,500
Molecular Weight:	17kDa
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 a gene product which had some interesting properties, including Calcium binding and the important observation that IBA1 was only expressed in hematopoietic cells (2). IBA1 and AIF1 were subsequently found to be identical, a small globular 17kDa molecule belonging to the “EF” hand superfamily of Calcium binding proteins. Since the only hematopoietic cells and in the neuropil of the central nervous system are microglia, suitable IBA1 antibodies are widely used to identify microglial cells in sections and tissues (3). In tissue samples from which they have not been washed out by perfusion, lymphocytes within blood vessels are also IBA1 positive. 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 (4). Many important and highly cited papers have made use of IBA1 antibodies as markers of microglia (e.g. 5,6). The IBA1 antibody was made against the C-terminal peptide of human IBA1 coupled to keyhole limpet hemocyanin. It works well on western blots, on cells cultures and sectioned material.
Form:	Liquid
Buffer:	Supplied as an aliquot of IgY preparation plus 5mM NaN3
Storage:	Store at 4°C

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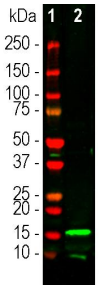
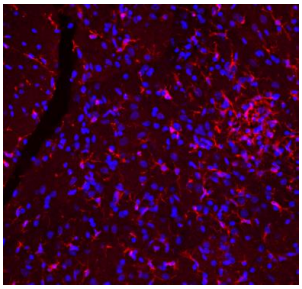


Western blot analysis of chicken pAb to IBA1, dilution 1:1,000 in green: [1] protein standard (red) and [2] rat spleen crude homogenate. The band at about 15kDa mark corresponds to IBA1 protein. IBA1 is a relatively minor protein of brain and is much more abundant in spleen, so the 15kDa band is less obvious in CNS lysates.



Rat spinal cord section, stained with IBA1, dilution 1:1,000, in red. Microglia are very small cells with fine processes spreading in three dimensions and so are best visualized in a confocal Z stack Nuclear DNA is shown with DAPI stain in blue.

Procedure for immunofluorescent staining of free-floating brain sections TISSUE PREPARATION:

• Perfuse the anesthetized animal (rat or mouse) transcardially with ice-cold PBS (pH=7.4), followed by freshly made 4% paraformaldehyde fixative solution in PBS. If you have never done an animal perfusion enlist help from a local experienced individual and make sure that you follow applicable local care of animals guidelines.
• Remove brain and other tissues and postfix in the same 4% paraformaldehyde fixative solution in PBS at 4°C for 16 – 24 hours.
• Cryoprotect the tissue by immersing in sucrose solutions in PBS, first 15% sucrose, for 24 hours then 30% sucrose until tissue sinks, which may take from 48 hours up to 1 week. Sinking indicates that the tissue is fully impregnated with sucrose and so cryoprotected.
• Cut 40 – 50µm sections on a cryostat.
• Store sections in PBS + 0.05M NaN3 at 4°C until they were taken for staining. Sections should never be allowed to dry out during storage or staining.

IMMUNOFLUORESCENT STAINING:

Rinse sections with PBS.
• Block and permeabilize sections in 10% normal goat serum or serum of the species the secondary antibodies were made in, in PBS plus 1% Triton-100 (PBSt) for 1 hour with slight agitation.
• Incubate sections with the primary antibody diluted in 1% normal goat serum in PBSt at 4°C overnight with slight agitation. Typical starting antibody concentration for pure antibody is 1mg/mL, though many will work well at much lower concentrations.
• Rinse sections 3 times with PBS, first rinse is quick, but wait 5 minutes between each subsequent rinses. This step removes unbound primary antibody.
• Add fluorochrome-conjugated secondary antibody, diluted 1:2,000, 0.5mg/mL, in 1% normal goat serum in PBSt. Can add Hoechst 33258 10mg/mL solution diluted 1:2,000, a convenient fluorescent blue dye for nuclear DNA staining. Cover specimen with foil and incubate for 2 hours at room temperature with slight agitation. We use Alexa Fluor. The ALEXA dyes are sulphonated rhodamine compounds and are more stable to UV than FITC, TRITC, Texas red etc. We typically incubate for 1hr at 37°C or 2 hours at room temperature or overnight at 4°C.
• Rinse sections 4 times with PBS, first rinse is quick, but wait 5-10 minutes between each subsequent rinse.
• Mount sections on clean glass slides with glycerol-base mounting medium, and cover with coverslip. Many vendors supply fluorescent sample mounting media which contain DAPI, a close relative of Hoechst 33258 mentioned in step 5 above. DAPI and Hoechst 33258 are both strong fluorescent DNA stains so if you use mounting media with DAPI you don’t need to add Hoechst in step 5 and vice versa. Store slides at 4°C, view on a suitable fluorescent, confocal or multiphoton microscope.

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