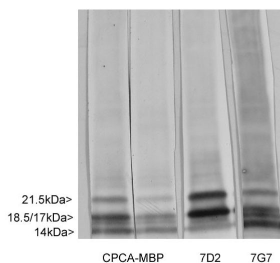
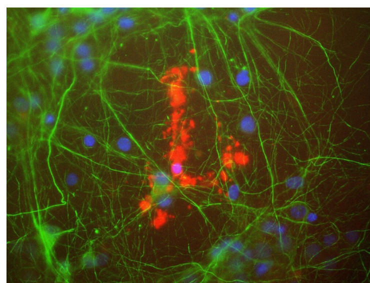
IMMUNOLOGICAL SCIENCES

Product Data Sheet: Myelin basic protein

Cat. No:	MAB-10660
Conjugate:	Unconjugated
Size:	100 ug
Clone:	7D2
Concentration:	1mg/ml
Host:	Ms
Isotype:	IgG1k
Immunogen:	Purified myelin basic protein isolated from cow nerve.
Reactivity:	Hu, Ms, Rt, Ct, Ch
Applications:	Western Blot: 1:1000 – 1: 5000 Immunofluorescence: 1:500 – 1: 2000 Immunocytochemistry: 1:500 – 1: 2000 Immunohistochemistry (frozen tissues): 1:500 – 1:2000
Molecular Weight:	14, 17, 18.5, and 21.5 kDa
Purification:	Purified
Background:	Myelin Basic Protein (MBP) is one of the major proteins of the myelin sheath surrounding axons in the nervous system. Since it is of relatively low molecular weight and high abundance the protein sequence was determined from purified protein over 30 years ago (1). The protein is made by oligodendrocytes in the central nervous system, so antibodies to MBP are good markers of this cell type. However, transcripts from the same gene are also expressed in certain hematopoetic lineage cells (2). In the mammalian central nervous system there are four different forms of the protein made by alternate transcription from a single gene, which have molecular weights of 21.5, 20.5, 18.5, and 17.2 kDa in humans. The single gene of rodents also produces 4 different proteins, but of slightly different sizes, 21.5, 18.5, 17 and 14 kDa. Sequence alignments of both sets of isotypes can be downloaded from here. We characterized our antibodies on rat spinal cord homogenates and detected the four bands expected for that species. As shown below, MAB-10660 shows preferential binding for the two higher molecular weight isoforms of 21.5 kDa and 18.5 kDa in the rat. The HGNC name for this protein is MBP.
Form:	Liquid
Buffer:	in PBS and glycerol (1:1) with 5mM NaN3
Storage:	Shipped at RT. Store at 4°C for short tem.for long term storage, store at -20°C. Avoid freeze / thaw cycles





Blots of crude rat spinal cord homogenate blotted with three MBP antibodies; (first two lanes) 7D2 (indicated lane) and (also as indicated). The 7D2 monoclonal binds the largest 21.5kDa and 18.5kDa transcripts preferentially, while 7G7 and MBP bind all four transcripts.

Rat mixed neuron/glial cultures stained with Myelin Basic Protein antibody 7D2 (red), and also with chicken antibody to neurofilament NF-L (green). Blue is a DNA stain. Note that the MBP antibody stains an oligodendrocyte and some membrane shed from this cell. Other cells in the field include neurons, astrocytes, microglia and fibroblasts, all of which are completely negative for MBP, though the neuronal processes can be seen with the NF-L antibody.

PROCEDURE OF IMMUNOFLUORESCENT STAINING OF FREE-FLOATING BRAIN TISSUE SECTIONS

TISSUE 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 secondary Antibody

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.