For Research Use Only.
Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

POLYCLONAL ANTIBODY

Anti-MBP (Myelin Basic Protein) pAb

Code No.	Quantity	Form
PD004	100 µL	Purified IgG

BACKGROUND: Myelin basic protein (MBP) is a hydrophilic, ~33 kDa homodimer that accounts for about 30 per cent of the proteins in the myelin sheath of the central nervous system, where it plays a role in both its formation and stabilization. At least 6 different MBP isoforms are produced by alternative splicing. MBP is expressed in oligodendrocytes, the myelin of white matter in the brain and spinal cord, and in peripheral nerves. It is expressed less abundantly in grey matter. MBP is the target of many post-translational modifications, including N-terminal acetylation, Arg methylation, phosphorylation by Ser/Thr kinases, and Gln deamidation. The incidence of MBP autoantibody in the autistic population is significantly higher than that of the normal population; hence, it serves as a primary marker of the autoimmune reaction in autism. Altered MBP levels and MBP autoantibodies are also associated with Multiple Sclerosis, Alzheimer's disease, Amyotrophic lateral sclerosis, and peripheral neuropathy.

SOURCE: This antibody is rabbit polyclonal antibody against the MBP peptide with the C-terminal cysteine extension (19-STMDHARHGFLP-30). The IgG was fractioned by salting out and gel filtration on a Sephadex G-200 column.

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human, mouse and rat MBP.

APPLICATIONS:
Western blotting; 1:200-1:1,000
Immunoprecipitation; Not recommended
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOL**.

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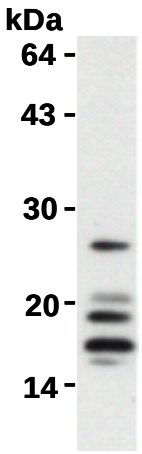
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Tissue		Brain	
Reactivity on WB	(+)*	+	(+)*

*The reactivity of this antibody to human and rat was not evaluated. However it is considered that this antibody reacts with human and rat as well as mouse because the immunogen sequence is completely conserved between human, mouse and rat.

REFERENCE:
1) Akiyama, K., *et al., J. Neurosci. Res.* **68**, 19-28 (2002)

This polyclonal antibody is used in this reference.



Western blot analysis of MBP expression in mouse brain using PD004.

PROTOCOL:
SDS-PAGE & Western blotting
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.

3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (MBL, code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; mouse brain)