

**Anti-phospho MBP**  
(mouse monoclonal IgG<sub>2a</sub>)  
Catalog # 05-429  
Lot # 18249

**Immunogen:** Phosphorylated synthetic peptide Thr98 corresponding to a human myelin basic protein sequence coupled to tuberculin and used to immunize BALB/c mice. Clone P12.

**Antibody Class:** IgG<sub>2a</sub>, produced by BALB/c mice. Splenocytes were propagated and fused with Sp2 myeloma cells and the resulting hybridoma clone was selected.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial prior to removing the cap.

**Formulation:** 100ng of protein A purified IgG in 100ml of 0.07M Tris-glycine, pH 7.4, 0.105M NaCl, 0.035% sodium azide with 30% glycerol. Liquid at -20°C.

**FOR IN VITRO RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS OR ANIMALS**

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**Quality Control Testing**

Immunoblot Analysis: 0.5-1µg/ml of this lot strongly detected phosphorylated MBP. **Note:** Some preparations of MBP contain basal levels of phosphorylated MBP, which are detected by the pMBP antibody.

Immunoprecipitation: Not tested.

Immunocytochemistry: Not tested

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**Additional Research Applications**

Immunohistochemistry: Not Tested.

Protein Kinase Assay: Phosphorylated MBP was detected in a direct kinase assay using the Non-Radioactive MAP Kinase Assay Kit (Catalog # 17-191) and an immunoprecipitation kinase assay was performed with the pMBP antibody using the Non-Radioactive MAPK Immunoprecipitation Kinase Assay Kit (Catalog # 17-192).

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**Background:** Myelin-specific basic protein (MBP, MW = 18,400) is a major component of the myelin sheath that coats neurons of the central nervous system. The exact physiological function of MBP remains unknown, although it has been implicated in the maintenance of the structural integrity of myelin. MBP is phosphorylated at five (and possibly more) sites *in vivo*.

*In vitro*, MBP is a substrate for phosphorylation by several different protein kinases such as MAP Kinase, cAMP-dependent protein kinase, calmodulin-dependent protein kinase, Protein Kinase C and Phosphorylase Kinase. Even highly specific protein kinases such as Raf1, MEK and MEKK can utilize MBP as an alternative substrate.

**References:**

1. Yon, M., *et al.*, J. Neuroimmuno. **58:** 121-129, 1995.
2. Yon, M., *et al.*, J. Neuroimmuno. **65:** 55-59, 1996.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (TBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-1ng/ml of a-phospho MBP**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat a-mouse** HRP conjugated IgG, 1:2000 dilution was used) in TBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).