



10 Old Barn Road · Lake Placid, NY 12946
Technical Support: T: 800 548-7853 · F: 518 523-4513
email: techserv@upstatebiotech.com
Sales Department: T: 800 233-3991 · F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstatebiotech.com

Certificate of Analysis

Anti-phospho MBP, clone P12
(mouse monoclonal IgG_{2a})
Catalog # 05-429
Lot # 21087

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_{2a}, 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: 100mg of protein A purified IgG in 100ml of PBS, pH 7.4 with 0.1% sodium azide. Frozen solution at -20°C.

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

Quality Control Testing

Immunoblot Analysis: 0.5-1µg/ml of this antibody 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

Additional Research Applications

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).

Immunohistochemistry: Not Tested.

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; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 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 **0.5-1mg/ml of anti-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 anti-mouse** HRP conjugated IgG, Catalog # 12-349, 1:3000 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).