



cell signaling solutions

Certificate of Analysis

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

Anti-phospho MBP, clone P12

(mouse monoclonal IgG_{2a})

Catalog # 05-429

Lot # 23852

Immunogen: Synthetic peptide corresponding to the human myelin basic protein sequence phosphorylated at Thr98 and coupled to tuberculin. Clone P12.

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: 100µg of protein A purified IgG_{2a} in 100µl of PBS, pH 7.4, 0.1% sodium azide and 30% glycerol. Liquid at -20°C.

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

Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot strongly detected phosphorylated MBP after incubation with MAP Kinase 2/Erk2, active (Catalog # 14-173). **Note:** Some preparations of MBP contain basal levels of phosphorylated MBP, which are detected by the pMBP antibody.

Immunoprecipitation: Not tested.

Immunocytochemistry: Please refer to reference 3.

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). An immunoprecipitation kinase assay was performed with a previous lot of this antibody using the Non-Radioactive MAP Kinase Immunoprecipitation Kinase Assay Kit (Catalog # 17-192).

References:

1. Yon, M., *et al.*, *J. Neuroimmuno.* **58**: 121-129, 1995.
2. Yon, M., *et al.*, *J. Neuroimmuno.* **65**: 55-59, 1996.
3. Mandell, JW and Gocan, N.C., *Anal. Biochem.* **293**: 264-268, 2001.

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 μ g/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 3% nonfat dry milk (Catalog # 20-200) in TBS (TBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-phospho-MBP, clone P12** 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, 1:2000 dilution was used) in TBS-MLK for 2 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).