



cell signaling solutions

Certificate of Analysis

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Anti-phospho-MBP, clone P12

(mouse monoclonal IgG_{2a})

Catalog # 05-429

Lot # 28676

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

Formulation: 100µg of protein A purified IgG_{2a} in 100µl of PBS, pH 7.4, 0.09% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

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

**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 EDTA; 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 TBS containing 3% nonfat dry milk (Catalog # 20-200), (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, 1:4000 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).