

Anti-phospho-MBP, clone BK403

(mouse monoclonal IgG_{1κ})

Catalog # 05-705

Lot # 24419

Immunogen: KLH-conjugated, synthetic peptide containing the sequence..PRpTPP...in which pT corresponds to phosphorylated threonine residue 98 of phosphorylated human MBP (myelin basic protein). The immunizing sequence is identical in pig, mouse, rat bovine

Specificity: Recognizes in-vitro phosphorylated myelin basic protein, Mr ~18-23kDa.

Species Cross-reactivity: Broad species cross reactivity expected based on sequence homology.

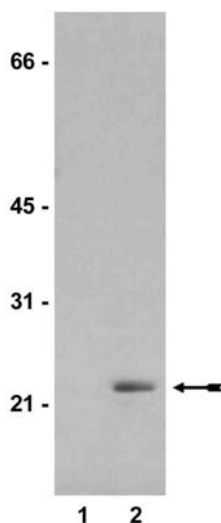
Formulation: 200μg of protein G purified mouse IgG_{1κ} in 232μl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

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

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.125-0.5μg/ml of this lot 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.



Immunoblot Analysis
MBP (Lane 1) and MBP phosphorylated *in vitro* with MAP Kinase 2/Erk2, active (Catalog # 14-173, Lane 2) were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-MBP, clone BK403 (0.125μg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates in-vitro phosphorylated MBP (18-23 kDa).

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 this lot of antibody using the Non-Radioactive MAP Kinase Immunoprecipitation Kinase Assay Kit (Catalog # 17-192).

General References:

1. Mandell, J.W. and N.C. Gocan, *Anal. Biochem.* **293**: 264-268, 2001.
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, pH 7.4; 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 with 0.05% Tween 20 (TBST-MLK) for one hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.125-0.5 μ g/ml of anti-phospho-MBP, clone BK403**, diluted in freshly prepared TBST-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, 1:5000 dilution was used) in TBST-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 10 minutes.
8. Rinse the nitrocellulose in water for 30 minutes.
9. Use detection method of choice (enhanced chemiluminescence was used).