



cell signaling solutions

Certificate of Analysis

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Anti-MBP, clone SKB3

(mouse monoclonal IgG_{1κ})

Catalog # 05-675

Lot # 22368

Immunogen: KLH-conjugated, synthetic peptide corresponding to the human myelin basic protein sequence containing amino acid Thr98.

Specificity: Recognizes MBP, Mr 18-20kDa.

Species Cross-reactivity: Human, mouse, and rat. Predicted to cross-react with bovine based on sequence homology.

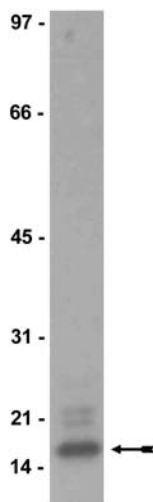
Formulation: 50µg of protein G purified mouse IgG_{1κ} in 50µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

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: .05-0.5µg/ml of this lot detected MBP in RIPA lysates from mouse and rat brain tissue preparations.



Immunoblot Analysis

Mouse brain cytosol homogenate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-MBP (0.1µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates MBP (18kDa).

Additional Research Applications

MAP Kinase Assay and Indirect ELISA: 0-200ng of MAP Kinase 2/Erk2, active (Catalog # 14-173) was used to phosphorylate well-bound MBP. Phosphorylation of MBP by MAP Kinase 2/Erk2, active was detected using anti-phospho-MBP (Catalog # 05-429). Total MBP was detected by anti-MBP (Catalog # 05-675).

General References:

1. Cicirelli, *et al.*, *J. Biol. Chem.* **263**: 2009, 1988.
2. Martenson, *et al.*, *J. Biol. Chem.* **258**: 930, 1983.
3. Ulmer, *et al.*, *J. Biol. Chem.* **262**: 1748, 1987.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a tissue protein preparation 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 PBS (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **.05-0.5 μ g/ml of anti-MBP**, diluted in freshly prepared PBS-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 PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-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).

Indirect ELISA Protocol

1. Rehydrate all wells of an MBP-coated plate (Catalog # 30-001) with 200 μ l of PBS and let stand for 15 minutes.
2. Aspirate PBS from all wells.
3. Block all wells with 200 μ l of 2% BSA/PBS for 1 hour.
4. Aspirate blocking buffer from all wells.
5. Add 100 μ l **anti-MBP (Catalog # 05-675)** diluted to 1:10,000 in 1% BSA/PBS to all wells of interest. Be sure to leave some wells incubating with blocking buffer alone, without the antibody, for negative control purposes.
6. Incubate plate for 1 hour at room temperature.
7. Wash all wells 5 times with PBS.
8. Add HRP-conjugated anti-mouse secondary antibody, 1:5000 dilution, to all wells and incubate plate for 45 minutes.
9. Wash all wells 5 times with PBS.
10. Add 75 μ l of horseradish peroxidase chemiluminescence substrate (Kirkegaard & Perry Laboratories) to all wells and read counts per second (CPS) using a chemiluminescence plate reader.