

---

## Certificate of Analysis

### Anti-phospho MBP, clone P12

(mouse monoclonal IgG<sub>2a</sub>)

Catalog # 05-429

Lot # 22887

**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<sub>2a</sub> in 100µl of PBS, pH 7.4, 0.1% sodium azide and 30% glycerol. Frozen solution at -20°C.

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

---

#### Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot 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). 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).

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; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 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 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 $\mu$ 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, 1:1000 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).