

**Myelin Basic Protein**  
**(bovine, ultra pure)**

**Catalog # 13-104**

**Lot # 16293**

**Background:** Myelin-specific basic protein (MBP, Mr = 18,400) is a major component of the myelin sheath that coats neurons of the central nervous system. The exact physiological function of MBP remains unknown, although it has been implicated in the maintenance of the structural integrity of myelin. MBP is phosphorylated at five (and possibly more) sites *in vivo*.

**Product Description:** *In vitro*, MBP is a substrate for phosphorylation by several different protein kinases such as MAP kinase, cAMP-dependent protein kinase, calmodulin-dependent protein kinase, protein kinase C and phosphorylase kinase. Even highly specific protein kinases such as Raf1, MEK and MEKK can utilize MBP as an alternative substrate.

**Purification:** CM-cellulose Chromatography, purity 95% by SDS-PAGE and Coomassie blue staining.

**Quantity and Formulation:** 10mg MBP in 4 vials, each vial containing 2.5mg MBP in 500µl of 10mM MOPS, pH 7.0, with 0.05% azide. Final concentration of 5mg/ml.

**Physical Form:** Frozen solution.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**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.

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

### Quality Control Testing and Research Applications

MAPK Assay: This lot was tested by using the recombinant active Erk2 MAPK (Catalog # 14-173) to phosphorylate MBP.

#### Kinase Assay Protocol

##### Stock Solutions:

1. Assay Dilution Buffer (ADB): 20mM MOPS, pH 7.2, 25mM  $\beta$ -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.
2.  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ : Stock 1mCi/100 $\mu\text{l}$  (3000Ci/mmol, obtained from DuPont-NEN). Make 10 $\mu\text{l}$  aliquots (100 $\mu\text{Ci}$ /vial). Before starting the assay, dilute an aliquot with 90 $\mu\text{l}$  of 500 $\mu\text{M}$  unlabeled ATP and 75mM  $\text{MgCl}_2$  in ADB. Final concentration = 1 $\mu\text{Ci}/\mu\text{l}$ .
3. Erk2/MAPK (Catalog # 14-173): Use 100ng per assay point.
4. Myelin Basic Protein (MBP): Dilute to 2.0mg/ml with ADB.

##### Assay Protocol:

1. Add 10 $\mu\text{l}$  of ADB to a microcentrifuge tube.
2. Add 10 $\mu\text{l}$  of Erk2 (100ng/assay).
3. Add 10 $\mu\text{l}$  of MBP (20 $\mu\text{g}$ ).
4. Add 10 $\mu\text{l}$  of the diluted  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ .
5. Incubate for 10 minutes at room temperature.
6. Spot 25 $\mu\text{l}$  onto the center of a 2cm x 2cm P81 paper.
7. Wash the assay squares three times with 0.75% phosphoric acid for 5 minutes each.
8. Wash the assay squares once with acetone for 5 minutes.
9. Transfer the assay squares to a scintillation vial and add 5ml scintillation cocktail.
10. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no enzyme (background control).