

## Myelin Basic Protein

(bovine, ultra pure)

Catalog # 13-104

Lot # 18674

**Product Description:** *In vitro*, myelin basic protein (MBP) is a substrate for phosphorylation by several different protein kinases including MAPK, PKA, calmodulin-dependent protein kinase, PKC and phosphorylase kinase. Even highly specific protein kinases such as Raf1, MEK and MEKK can utilize MBP as an alternative substrate.

**Purification** SP-Sepharose™ high performance liquid chromatography to a purity of 95% as judged after SDS-PAGE and Coomassie blue staining of 1µg of product.

**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 after thawing and prior to removing the cap.

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

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NOT FOR USE IN HUMANS**

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### Quality Control Testing

**Protein Kinase Assay:** This lot was successfully phosphorylated using MAP Kinase 2/Erk2, active (Catalog # 14-173) in a kinase assay.

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**Background:** Myelin-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*.

#### 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.

### 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. [ $\gamma$ -<sup>32</sup>P]ATP: Stock 1mCi/100 $\mu$ l (3000Ci/mmol, obtained from DuPont-NEN). Make 10 $\mu$ l aliquots (100 $\mu$ Ci/vial). Before starting the assay, dilute an aliquot with 90 $\mu$ l of 500 $\mu$ M unlabeled ATP and 75mM MgCl<sub>2</sub> in ADB. Final concentration = 1 $\mu$ Ci/ $\mu$ l.
3. MAPK2/Erk2 (Catalog # 14-173): Use 25ng/10 $\mu$ l per assay point.
4. Inhibitor Cocktail (Catalog # 20-116): 20 $\mu$ M PKC inhibitor peptide [Catalog # 12-121], 2 $\mu$ M PKA inhibitor peptide (PKI) [Catalog # 12-151] and 20 $\mu$ M Compound R24571 in ADB .
5. Myelin Basic Protein (MBP): Dilute to 2mg/ml with ADB. Use 20 $\mu$ g/10 $\mu$ l per assay point.

#### Assay Protocol:

1. Add 10 $\mu$ l of ADB to a microcentrifuge tube.
2. Add 10 $\mu$ l of MAPK2 (25ng).
3. Add 10 $\mu$ l of MBP (20 $\mu$ g).
4. Add 10 $\mu$ l of the inhibitor cocktail or ADB.
5. Add 10 $\mu$ l of the diluted [ $\gamma$ -<sup>32</sup>P]ATP.
6. Incubate for 10 minutes at room temperature.
7. Spot 25 $\mu$ l onto the center of a 2cm x 2cm P81 paper.
8. Wash the assay squares three times with 0.75% phosphoric acid for 5 minutes each.
9. Wash the assay squares once with acetone for 5 minutes.
10. Transfer the assay squares to a scintillation vial and add 5ml scintillation cocktail.
11. Read in scintillation counter. Compare CPM of enzyme samples to CPM of control samples that contain no enzyme (background control).