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Certificate of Analysis

MBP, bovine, purified (myelin basic protein) Catalog # 13-104 Lot # 20761

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 500ml of 10mM MOPS, pH 7.0, with 0.05% sodium azide. Final concentration: 5mg/ml. Frozen solution.

Sepharose™ is a trademark of Pharmacia Biotech.

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

Quality Control Testing

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

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 β -glycerol phosphate, 5mM EGTA, 1mM sodium orthovanadate, 1mM dithiothreitol.
2. [γ - 32 P]ATP: Stock 1mCi/100 μ l (3000Ci/mmol, obtained from DuPont-NEN). Make 10 μ l aliquots (100 μ Ci/vial). Before starting the assay, dilute an aliquot with 90 μ l of 500 μ M unlabeled ATP and 75mM MgCl₂ in ADB. Final concentration = 1 μ Ci/ μ l.
3. MAPK2/Erk2 (Catalog # 14-173): Just prior to the assay, dilute with ADB to 2.5ng/ μ l. Use 25ng per assay point.
4. Inhibitor Cocktail (Catalog # 20-116): 20 μ M PKC inhibitor peptide [Catalog # 12-121], 2 μ M PKA inhibitor peptide (PKI) [Catalog # 12-151] and 20 μ M Compound R24571 in ADB .
5. Myelin Basic Protein (MBP): Dilute to 2mg/ml with ADB. Use 10 μ l per assay point.

Assay Protocol:

1. Add 10 μ l of ADB to a microcentrifuge tube.
2. Add 10 μ l of MAPK2 (25ng).
3. Add **10 μ l of MBP** (20 μ g).
4. Add 10 μ l of the inhibitor cocktail or ADB.
5. Add 10 μ l of the diluted [γ - 32 P]ATP.
6. Incubate for 10 minutes at 30°C.
7. Spot 25 μ 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).