



cell signaling solutions

Certificate of Analysis

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MBP, bovine, purified

(myelin basic protein)

Catalog # 13-104

Lot # 23514

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.

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 MAP Kinase 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 I (ADBI, Catalog # 20-108): 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 ADBI. Final concentration = 1 μ Ci/ μ l.
3. MAP Kinase2/Erk2, active (Catalog # 14-173): Dilute to 2.5ng/ μ l with ADBI. Use 25ng per assay point. Dilute just prior use.
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 ADBI.
5. MBP (Myelin Basic Protein): Dilute to 2mg/ml with ADBI. Use 10 μ l per assay point.

Assay Protocol:

1. Add 20 μ l of ADBI to a microcentrifuge tube.
2. Add 10 μ l (25-100ng) of MAP Kinase 2/Erk2, active per assay point.
3. Add 10 μ l (20 μ g) of **MBP, bovine, purified**.
4. Add 10 μ l of the diluted [γ - 32 P]ATP.
5. If necessary, add ADBI to bring the volume to 50 μ l.
6. Incubate for 15 minutes at 30°C with rigorous agitation.
7. Transfer 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).

Technical Note: Allow the radiolabeled substrate to bind to the filter paper for 30 seconds before immersing the paper into a 50ml conical tube containing 40ml 0.75% phosphoric acid. Gently shake the assay squares for 5 minutes on a rotator. Discard the wash in a liquid radioisotope waste container, (dispose of per institutional regulations) and repeat the wash step twice. Wash the squares in 20ml of acetone for 5 minutes. Drain and add scintillation cocktail.