

**Certificate of Analysis**

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**MBP, bovine, purified**  
(myelin basic protein)  
Catalog # 13-104  
Lot # DAM1493361

**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:** Purified by HPLC to 95% as determined by SDS-PAGE and Coomassie blue staining of 1µg of product.

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

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

**Handling Recommendations:** Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles.**

**FOR RESEARCH USE ONLY  
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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**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  $\beta$ -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 PerkinElmer, Cat. # BLU002A). 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 ADBI. Final concentration = 1 $\mu$ Ci/ $\mu$ l.
3. MAP Kinase 2/Erk2, active (Catalog # 14-173): Dilute to 2.5ng/ $\mu$ l with ADBI. Use 25ng per assay point. Dilute just prior use.
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 ADBI.
5. MBP (Myelin Basic Protein): Dilute to 2mg/ml with ADBI. Use 10 $\mu$ l per assay point.

#### Assay Protocol:

1. Add 10 $\mu$ l of ADBI to a microcentrifuge tube.
2. Add 10 $\mu$ l of Inhibitor Cocktail.
3. Add 10 $\mu$ l (25-100ng) of MAP Kinase 2/Erk2, active per assay point.
4. Add 10 $\mu$ l (20 $\mu$ g) of **MBP, bovine, purified**.
5. Add 10 $\mu$ l of the diluted [ $\gamma$ -<sup>32</sup>P]ATP.
6. If necessary, add ADBI to bring the volume to 50 $\mu$ l.
7. Incubate for 15 minutes at 30°C with rigorous agitation.
8. Transfer 25 $\mu$ l onto the center of a 2cm x 2cm P81 paper.
9. Wash the assay squares three times with 0.75% phosphoric acid for 5 minutes each.
10. Wash the assay squares once with acetone for 5 minutes.
11. Transfer the assay squares to a scintillation vial and add 5ml scintillation cocktail.
12. 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.

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