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Certificate of Analysis

MBP, recombinant

(recombinant protein expressed in *E. coli*)
Catalog # 13-173
Lot # XXXXXXXXXXX

Description: Recombinant fusion protein containing bovine myelin basic protein (MBP) with an N-terminal GST tag and a C-terminal 6His tag, expressed in *E. coli.*

Accession Number: P02687

Purity: ~15% by SDS-PAGE and Coomassie blue staining.

Molecular Weight: ~47 kDa.

Formulation: 500 µg MBP recombinant in **500 µL** of 50mM Tris-HCl, pH 8.0, 150mM NaCl, 20mM GSH, 1mM PMSF, and 10% glycerol. Frozen solution.

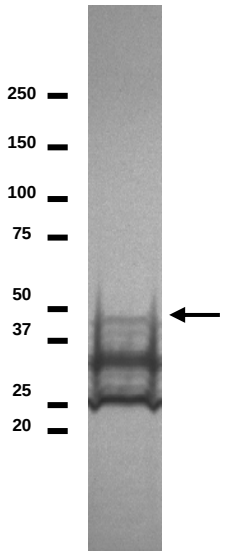
Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

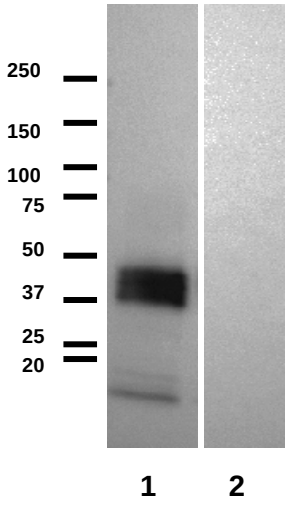
FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Quality Control Testing

SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 5µg of MBP-GST, Arrow indicates MBP-GST (47 kDa).



Non-radioactive kinase assay: This lot was successfully phosphorylated in a non-radioactive kinase assay using recombinant active MAP Kinase 2/Erk2 (Catalog # 14-173). MBP phosphorylation was detected by immunoblot analysis using anti-phospho-MBP (Catalog # 05-429). Immunoblot shown below.



Immunoblot Analysis

250 ng of recombinant MBP (lanes 1 and 2) were incubated with (lane 1) or without (lane 2) MAP Kinase 2/Erk2 (Catalog # 14-173), resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-MBP (Catalog # 05-429, 1 µg/mL). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

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Kinase Assay Procedure

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. **Magnesium/ATP Cocktail** (Catalog # 20-113): 500μM unlabeled ATP and 75mM $MgCl_2$ in ADBI.

3. **MAP Kinase 2/Erk2, active** (Catalog # 14-173): Dilute to 10-20 ng/μl. Use 10 μl per assay point.

4. **Recombinant MBP:** Dilute to 1 mg/ml with ADBI. Use 4-10 μl per assay point.

5. **Anti-phospho MBP** (Catalog # 05-429): Use 1 μg/ml per immunoblot assay.

6. **Goat-anti-mouse HRP conjugated IgG**, (Catalog # 12-349)

Example Non-Radioactive Kinase Assay Protocol

1. Add 16μl of ADBI to a microcentrifuge tube.
2. Add 10μl of MAP Kinase 2/Erk2, active (100ng).
3. Add 4μl of recombinant MBP (4μg).
4. Add 10μl of Mg^{2+}/ATP Cocktail.
5. Incubate for 30 minutes at 30°C with constant shaking.
6. Add 40μl of 2X Reducing Sample Buffer (RSB) to the reaction mixture.
7. Perform SDS-Page (5μl, or 250ng per lane) transfer the proteins to nitrocellulose membranes.
8. Block the blotted nitrocellulose in freshly prepared PBS containing 5% nonfat dry milk and 0.05% Tween-20 (PBST-MLK) for 20 minutes at room temperature with constant agitation.
9. Incubate the nitrocellulose with 1μg/ml of anti-phospho MBP (Catalog # 05-429) diluted in freshly prepared PBST-MLK overnight with agitation at 4°C.
10. Wash the nitrocellulose twice with water.
11. Incubate the nitrocellulose in the secondary reagent of choice (a goat-anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:3000 dilution was used) in PBST-MLK for 1.5 hours at room temperature with agitation.
12. Wash the nitrocellulose with water twice.
13. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
14. Rinse the nitrocellulose in 4-5 changes of water.
15. Use detection method of choice (enhanced chemiluminescence was used).

Recombinant MBP Sequence Information

Protein **Bovine Myelin Basic Protein**

Tags **N-terminal GST**

Native sequence **A227 of the fusion protein is equivalent to A1 of bovine MBP.**

Accession number **GenBank # P02687**

Recombinant bovine MBP amino acid sequence:

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  1  MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID
 61  GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV
121  DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK
181  KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSAAQK RPSQRSKYLA
241  SASTMDHARH GFLPRHRDTG ILDSLGRFFG SDRGAPKRGS GKDGHHAART THYGSLPQKA
301  QGHRPQDENP VVHFFKNIVT PRTPPPSQGK GRGLSLSRFS WGAEGQKPGF GYGGRASDYK
       361  SAHKGLKGHD AQGTLSKIFK LGGRDSRSGS PMARRKGELI RRAGTHHHHH H
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