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ProductInformation

MONOCLONAL ANTI-MALTOSE BINDING PROTEIN (MBP)
CLONE MBP-17
Mouse Ascites Fluid

Product Number **M 6295**

Product Description

Monoclonal Anti-Maltose Binding Protein (MBP) (mouse IgG1 isotype) is derived from the MBP-17 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a purified recombinant MBP fusion protein. The isotype is determined using Sigma ImmunoType Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal anti-Maltose Binding Protein (MBP), recognizes native as well as denatured-reduced forms of purified MBP or MBP fusion proteins, applying immunoblotting, dot blot and ELISA.

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes that can provide 'affinity handles' (tags) designed to enable the selective identification and purification of the protein of interest.[1-3] These sequences of tails or tags are genetically engineered away from the protein active site, by insertion at the N- or C-terminus.

It has been reported that the addition of a maltose binding protein (MBP) tag creates a stable fusion product that does not appear to interfere with the bioactivity of the protein or with the biodistribution of the MBP tagged product.[4,5] The expression of polypeptides in-frame with maltose binding protein (MBP) allows for their easy purification from bacterial extracts under mild conditions, which employ a single affinity chromatographic step on amylose resin.[4] This system and others based on the expression of fusion proteins utilize a specific protease cleaving site to facilitate correct cleavage of the fusion protein.[3] Thus, the MBP system incorporates a factor Xa cleavage site at the carboxy terminus of the MBP sequence,[5] and cleavage by factor Xa separates MBP from its partner protein. Many recombinant proteins[4-6] have been engineered with MBP tags to facilitate the detection, isolation and purification of the proteins. Monoclonal antibody reacting specifically with MBP may be useful in various immunotechniques, to identify the expression of a MBP fusion protein in bacteria, bacterial lysates or cells and tissues transfected with a MBP fusion protein expressing vectors.

Reagent

Monoclonal Anti-Maltose Binding Protein is provided as ascites fluid with 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Solutions at working dilution should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:4,000 is determined by immunoblotting, using purified recombinant MBP.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

Procedure

Immunoblotting

All incubation steps should be performed at room temperature.

1. Separate MBP-tagged proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5 to 20 µg of total lysate protein per lane. The amount of lysate to be loaded per lane depends on the level of protein expression and may vary between experiments.

2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5 % non-fat dry milk in phosphate buffered saline (PBS, Product No. D 8537) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20 (Product No. P 3563).
5. Incubate the membrane with Anti-Maltose Binding Protein (MBP) using an optimized concentration in PBS containing 1 % bovine serum albumin (BSA, Product No. A 9647) for two hours.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20.
7. Incubate the membrane with Anti-mouse IgG Peroxidase conjugate (e.g. Product No. A 9917, A 3682, or A 2304) or with Anti-mouse Alkaline Phosphatase conjugate (e.g. Product No. A 1293, A 2179 or A 1682) as the secondary antibody at the recommended concentration in PBS containing 0.05 % Tween 20. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20.
9. Treat the membrane with either a peroxidase or an alkaline-phosphatase substrate as appropriate.

References

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4. Guan, C., et al., Gene, **67**, 21-30 (1988).
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