3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Monoclonal Anti-Maltose Binding Protein–Alkaline Phosphatase, clone MBP-17
produced in mouse, purified immunoglobulin

Catalog Number **A3963**

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 purified recombinant MBP fusion protein. The immunoglobulin fraction of antibody to Maltose Binding Protein is purified from ascites fluid of the MBP-17 hybridoma and then conjugated to calf intestinal alkaline phosphatase using glutaraldehyde.

Monoclonal Anti-Maltose Binding Protein–Alkaline Phosphatase recognizes native as well as denatured-reduced forms of purified MBP or MBP fusion proteins by immunoblotting, dot blotting or 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 bio-distribution 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

Supplied as a solution in 0.05 M Tris buffer pH 8.0, containing 1 % BSA, 1 mM $MgCl_2$, 50 % glycerol and 15 mM sodium azide. The specific antibody concentration is at least 1 mg/ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use and extended storage, store at 2-8 °C. Do not freeze. Solutions at working dilution should be discarded if not used within 12 hours.

Product Profile

A minimum working dilution of 1:400 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 SDS-PAGE protocol. Load 2.5 to 20 μg of total lysate protein per lane. Note: 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 for at least 60 minutes using a solution of 5 % non-fat dry milk in phosphate buffered saline (PBS, Catalog Number D8537).
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % TWEEN® 20, Catalog Number P3563.
5. Incubate the membrane with Monoclonal Anti-Maltose Binding Protein-Alkaline Phosphatase using an optimized concentration in PBS containing 1 % bovine serum albumin (BSA, Catalog Number A9647) for two hours.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % TWEEN 20.
7. Treat the membrane with an alkaline phosphatase substrate (e.g. BCIP/NBT, Catalog Number B1911).

References

1. Narayanan, S.R., *J. Chromatogr*., **658**,237-258 (1994).
2. Casey, J.L., et al., *J. Immunol. Meth*., **179**, 105-116 (1995).
3. Uhlen, M., and Moks, T., *Meth. Enzymol*., **185**, 129-143 (1990).
4. Guan, C., et al., *Gene*, **67**, 21-30 (1988).
5. Maina, C.V., et al., *Gene*, **74**, 365-373 (1988).
6. Rodriguez, P.L., and Carrasco, L., *Biotechniques*, **18**, 238-243 (1995).

TWEEN is a registered trademark of Uniqema Americas LLC

DS,KAA,SN,PHC 10/11-1