Product Information

# Monoclonal Anti-Maltose Binding

Protein-Peroxidase Antibody Produced in Mouse
Clone MBP-17, Purified Immunoglobulin, Lyophilized Powder

**A4213**
Storage Temperature 2-8 °C

## 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 with horseradish peroxidase (HRP).

Monoclonal anti-Maltose Binding Protein (MBP), Peroxidase conjugate, 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 insertion of genes of interest, to specific sequences or genes, which 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 chromato-graphic 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 (MBP), Peroxidase conjugate is supplied as lyophilized powder. After reconstitution the solution contains 1% BSA and 0.05% MIT in 0.01 M sodium phosphate buffered Saline (PBS).

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| Antibody concentration: | 5 to 11 mg/mL |
| Molar ratio Ab/Enzyme: | 0.8 to 1.5 |
| Enzyme activity | At least 400 U/mL |

## Preparation Instructions

Reconstitute the contents of the vial with 0.5 mL of distilled water.

## Storage/Stability

For extended storage after reconstituting the lyophilized conjugate in distilled water, store at −20 °C in working aliquots. Avoid repeated freeze-thaw cycles. Do not store in frost-free freezers. For continuous use after reconstitution, keep between 2 and 8 °C for up to 1 month. Solutions at working dilution should be discarded if not used within 12 hours.

## Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Product Profile

A minimum working dilution of 1:1,000 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, Cat. No. D8537) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween® 20 (Cat. No. P3563).
5. Incubate the membrane with Anti-Maltose Binding Protein (MBP), Peroxidase conjugate using an optimized concentration in PBS containing 0.05% Tween® 20 and 1% bovine serum albumin (BSA, Cat. No. 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 a peroxidase substrate. (such as, AEC, Cat. No. AEC-101).

## References


1. Narayanan, S.R., J. Chromatogr., **658**: 237-258 (1994).
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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).


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