

MONOCLONAL ANTIBODY

# Anti-MBP (Maltose Binding Protein) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M091-3	1G12	Mouse IgG3	100 µL	1 mg/mL

**BACKGROUND:** Maltose Binding Protein (MBP) is a part of the maltose/maltodextrin system of *Escherichia coli* bacteria, which is responsible for the uptake and efficient catabolism of maltodextrins. It is a complex regulatory and transport system involving many proteins and protein complexes. MBP is often used as a fusion partner for recombinant protein expression, primarily because its affinity for maltodextrins allows for facile purification of fusion proteins by amylose affinity chromatography.

**SOURCE:** This antibody was purified from hybridoma (clone 1G12) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse lymphocyte immunized with recombinant MBP.

**FORMULATION:** 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at -20°C.

**REACTIVITY:** This antibody reacts with MBP on Western blotting and Immunoprecipitation.

## APPLICATIONS:

Western blotting; 1 µg/mL  
Immunoprecipitation; 2 µg  
Immunohistochemistry; Not tested  
Immunocytochemistry; Not tested  
Flow cytometry; Not tested

Detailed procedure is provided in the following **PROTOCOLS**.

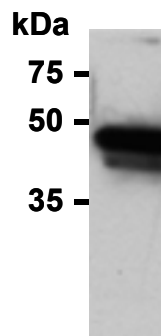
## INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

## REFERENCES:

- 1) Kasahara, K., *et al.*, *Nat. Commun.* **5**, 5081 (2014) [IP]
- 2) Ito, T., *et al.*, *Plant Cell* **22**, 1592-1604 (2010) [WB]

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

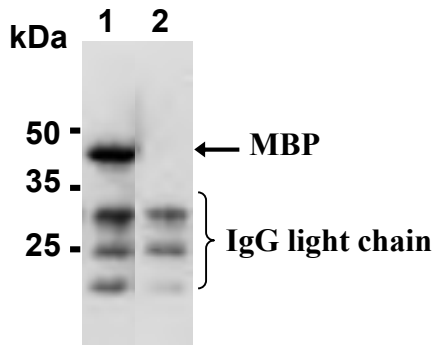


**Western blot analysis of MBP expression in pMAL-c2G/JM109 *E. coli* lysate using M091-3.**

## PROTOCOLS:

### SDS-PAGE & Western blotting

- 1) Mix the sample with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on conditions.)
- 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
- 7) Incubate the membrane with 1:10,000 Anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 8) Wash the membrane with PBS-T (5 minutes x 6).
- 9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 10) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.



**Immunoprecipitation of MBP from pMAL-c2G/JM109 E. coli lysate with M091-3 (1) or mouse IgG3 (2) . After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M091-3.**

### **Immunoprecipitation**

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggested in the **APPLICATIONS** into 100 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 5) Resuspend the agarose with cold Lysis buffer.
- 6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 7) Repeat steps 5)-6) 2-4 times.
- 8) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 µL/lane for the SDS-PAGE analysis.  
(See **SDS-PAGE & Western blotting.**)

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