Anti-MBP Antiserum

1-800-632-7799
info@neb.com
www.neb.com

E8030S 086120314031

E8030S

0.2 ml **Lot: 0861203**

Store at –20°C **Exp: 3/14**

Description: Rabbit antiserum prepared by immunizing against affinity-purified maltose-binding protein.

Source: Serum from rabbits immunized with maltose-binding protein

Specificity: Tested by Western blot and ELISA assay. Reacts specifically with maltose-binding protein.

Suggested Working Dilution: 1/10,000.

Performance: In an ELISA assay, a dilution of 1/10,000 added to a microtiter plate containing 1 µg purified maltose-binding protein per well gives a value of approximately 1.0 at OD490 after 8 minutes reaction time, using OPD as the substrate for horseradish peroxidase. The same dilution gives a strong signal when used to detect maltose-binding protein in Western blots developed with a variety of detection systems. Once diluted, the serum may be reused a few times in Western blots.

Western Transfer Protocol

Materials:
Transfer apparatus and associated buffers
Nitrocellulose or PVDF membrane
TBST (20 mM Tris-Cl, 150 mM NaCl, 0.1% Tween 20)
Blocking Buffer (TBST + 5% Nonfat Dry Milk)
Anti-MBP Antiserum NEB #E8030
anti-rabbit antibody conjugated to peroxidase
Detection reagent

For a 10 cm x 10 cm gel:

1. Transfer protein from the gel to a nitrocellulose or PVDF membrane following the directions of the transfer apparatus manufacturer. Mark the wells of the gel on the filter with a blunt pencil before removing and discarding the gel.
2. Rinse the membrane with TBST.
3. Incubate the membrane with 25 ml Blocking Buffer for 1 hour at room temperature (or overnight at 4°C) with gentle shaking.
4. Wash the membrane in 25 ml TBST at room temperature with gentle shaking, 3 times for 5 minutes each.
5. Add 1 µl of the Anti-MBP Antiserum to 10 ml Blocking Buffer (a 1:10,000 dilution). Cover the membrane with the antibody dilution and incubate for 1 hour at room temperature with gentle shaking.
6. Wash the membrane in 25 ml TBST at room temperature with gentle shaking, 3 times for 5 minutes each.
7. Make a dilution of an anti-rabbit IgG-peroxidase conjugate in 10 ml Blocking Buffer according to the manufacturer's recommendation, and incubate the membrane in the solution for 1 hour.
8. Wash the membrane in 25 ml TBST at room temperature with gentle shaking, 3 times for 5 minutes each.
9. Follow the manufacturer's directions for detection.

Note: Store at –20°C undiluted. May be stored at 4°C diluted in buffer containing 1 mM NaN_3.

CERTIFICATE OF ANALYSIS

Anti-MBP Antiserum

1-800-632-7799
info@neb.com
www.neb.com

E8030S 086120314031

E8030S

0.2 ml **Lot: 0861203**

Store at –20°C **Exp: 3/14**

Description: Rabbit antiserum prepared by immunizing against affinity-purified maltose-binding protein.

Source: Serum from rabbits immunized with maltose-binding protein

Specificity: Tested by Western blot and ELISA assay. Reacts specifically with maltose-binding protein.

Suggested Working Dilution: 1/10,000.

Performance: In an ELISA assay, a dilution of 1/10,000 added to a microtiter plate containing 1 µg purified maltose-binding protein per well gives a value of approximately 1.0 at OD490 after 8 minutes reaction time, using OPD as the substrate for horseradish peroxidase. The same dilution gives a strong signal when used to detect maltose-binding protein in Western blots developed with a variety of detection systems. Once diluted, the serum may be reused a few times in Western blots.

Western Transfer Protocol

Materials:
Transfer apparatus and associated buffers
Nitrocellulose or PVDF membrane
TBST (20 mM Tris-Cl, 150 mM NaCl, 0.1% Tween 20)
Blocking Buffer (TBST + 5% Nonfat Dry Milk)
Anti-MBP Antiserum NEB #E8030
anti-rabbit antibody conjugated to peroxidase
Detection reagent

For a 10 cm x 10 cm gel:

1. Transfer protein from the gel to a nitrocellulose or PVDF membrane following the directions of the transfer apparatus manufacturer. Mark the wells of the gel on the filter with a blunt pencil before removing and discarding the gel.
2. Rinse the membrane with TBST.
3. Incubate the membrane with 25 ml Blocking Buffer for 1 hour at room temperature (or overnight at 4°C) with gentle shaking.
4. Wash the membrane in 25 ml TBST at room temperature with gentle shaking, 3 times for 5 minutes each.
5. Add 1 µl of the Anti-MBP Antiserum to 10 ml Blocking Buffer (a 1:10,000 dilution). Cover the membrane with the antibody dilution and incubate for 1 hour at room temperature with gentle shaking.
6. Wash the membrane in 25 ml TBST at room temperature with gentle shaking, 3 times for 5 minutes each.
7. Make a dilution of an anti-rabbit IgG-peroxidase conjugate in 10 ml Blocking Buffer according to the manufacturer's recommendation, and incubate the membrane in the solution for 1 hour.
8. Wash the membrane in 25 ml TBST at room temperature with gentle shaking, 3 times for 5 minutes each.
9. Follow the manufacturer's directions for detection.

Note: Store at –20°C undiluted. May be stored at 4°C diluted in buffer containing 1 mM NaN_3.

U.S. Patent No. 5,643,758
PCT Publication No W02007/120809
The Buyer/User has a non-exclusive license to use this vector for RESEARCH PURPOSES ONLY. Commercial use requires a license form New England Biolabs, Inc.

CERTIFICATE OF ANALYSIS