**Anti-MBP Antiserum**

**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

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.

**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.

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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.

**Note:** Store at –20°C undiluted. May be stored at 4°C diluted in buffer containing 1 mM NaN₃.

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**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.

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3. Incubate the membrane with 25 ml Blocking Buffer for 1 hour at room temperature (or overnight at 4°C) with gentle shaking.
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