Anti-LexA Antibody

Catalog no. R990-25

Version B
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Overview

Introduction

The Anti-LexA Antibody allows detection of LexA protein and recombinant proteins fused to the LexA protein. This antibody can be used to detect expression of potential bait proteins constructed for use with Hybrid Hunter™ Two-Hybrid System or other interaction trap systems (Gyuris et al., 1993).

Features of the LexA Protein

LexA adds about 22 to 25 kDa to your protein, depending on the number of extra amino acids. In experiments at Invitrogen, we have observed that the LexA protein expressed from pHybLex/Zeo (calculated molecular weight of 26 kDa), migrates at ~32 kDa.

Contents

50 microliters of Anti-LexA rabbit polyclonal antibody is supplied in PBS containing 0.01% sodium azide (added as a preservative). Please refer to the Material Safety Data Sheet (MSDS) for safety information (see page 5). Enough antibody is provided to perform 25 Western (immuno) blots based on information in the Certificate of Analysis.

Shipping/Storage

The Anti-LexA Antibody is shipped and stored at +4°C. Repeated freezing and thawing is not recommended as it may result in loss of antibody activity.

Specificity of the Antibody

The Anti-LexA Antibody has been tested in immunoblotting procedures using yeast lysates and purified protein. In Western blot experiments with purified protein, 500 ng of recombinant protein gave a strong signal using both chemiluminescent and alkaline phosphate detection reagents.

Recommended Dilution

For Western blots, dilute the Anti-LexA Antibody 1:5000 into Tris-Buffered Saline (TBS) containing 0.05% Tween-20 and 1% bovine serum albumin (BSA) or PBS containing 1% (w/v) BSA immediately before use.

If you use a different buffer for washing and blocking your blots, then dilute the antibody into that buffer. You may use other blocking agents such as gelatin or dry milk powder.

If you use alkaline phosphatase-conjugated secondary antibody, do not use PBS. Phosphate inhibits alkaline phosphatase. Use TBS instead.

If you use horseradish peroxidase (HRP)-conjugated secondary antibody, be sure to wash the Western blot or microtiter wells thoroughly before adding the color development solution. Azide in the antibody buffer will inhibit horseradish peroxidase. In addition, use 2% dry milk instead of BSA to prevent high background levels.
**Western Blot (Immunoblotting)**

**Introduction**

This procedure can be used for detection of fusion protein expression. We have included a general protocol for your convenience. The table below outlines the basic steps of a Western blot.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
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<tbody>
<tr>
<td>1</td>
<td>Electrophorese cell lysate or purified or partially purified protein with appropriate controls on an SDS polyacrylamide gel.</td>
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<tr>
<td>2</td>
<td>Transfer the proteins electrophoretically to a nylon or nitrocellulose membrane.</td>
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<tr>
<td>3</td>
<td>Block membrane to reduce background.</td>
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<tr>
<td>4</td>
<td>Probe the blot with Anti-LexA Antibody.</td>
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<tr>
<td>5</td>
<td>Incubate the blot with appropriate secondary antibodies for chemiluminescent, alkaline phosphatase, or HRP detection.</td>
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<tr>
<td>6</td>
<td>Generate signal using the appropriate detection reagents.</td>
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</table>

**Detection Reagents Required**

We use chemiluminescent or alkaline phosphatase reagents to detect binding of the Anti-LexA Antibody to recombinant proteins. Other detection methods can be used to detect your protein.

Chemiluminescent Reagents (please refer to manufacturer's instructions)

Alkaline Phosphatase Reagents

- Secondary Antibody: Anti-Rabbit Immunoglobulin Alkaline Phosphatase conjugate (Sigma-Aldrich A2306)
- Enzyme Substrates for Alkaline Phosphatase: Bromochloroindolyl phosphate (BCIP) and nitro blue tetrazolium (NBT). Prepare 10 ml of a 50 mg/ml stock solution of each substrate. Use 100% dimethylformamide (DMF) to dissolve BCIP and 70% DMF to dissolve NBT.
- Alkaline phosphatase buffer (100 mM diethanolamine, 100 mM NaCl, 5 mM MgCl₂, pH 9.5)

**Reagents for Immunoblotting**

The following materials and solutions are needed for immunoblotting. Dry milk may be substituted for BSA at a final concentration of 2% for both the blocking buffer and the dilution buffer.

- Tris-Buffered Saline (TBS: 20 mM Tris-HCl, 500 mM NaCl, pH 7.5)
- Tris-Buffered Saline + Tween 20 (TBST: TBS plus 0.05% Tween-20, w/v)
- Bovine Serum Albumin (BSA), powdered
- Blocking buffer (TBS + 3% BSA, w/v)
- Dilution buffer (TBST + 1% BSA, w/v)

*Continued on next page*
Western Blot, continued

Immunoblotting Protocol

Prepare an SDS polyacrylamide gel (either Tris/Glycine or Tris/Tricine) designed to resolve your recombinant protein. Prepare your samples for electrophoresis. (For information about SDS-polyacrylamide gel electrophoresis, please see Ausubel, et al., 1990.)

1. Load your samples and electrophorese your SDS polyacrylamide gel.
2. Prepare Transfer Buffer: 25 mM Tris, 192 mM glycine, 20% v/v methanol pH 8.3 (or your buffer of choice).
3. Transfer proteins to nitrocellulose (or other appropriate membrane) electrophoretically using the settings recommended by the manufacturer of your transfer apparatus.
4. Remove blot and incubate it in 10 to 20 ml blocking buffer. Gently agitate using a rocker platform for 1 hour at room temperature.
5. Wash blot in 20 ml TBST for 5 minutes with gentle agitation. Repeat wash once more.
6. Add Anti-LexA Antibody diluted as described in the Certificate of Analysis in 10 ml dilution buffer. Incubate with gentle agitation for 2 hours at room temperature. (Overnight incubation at +4°C may be preferred, since longer incubations may increase sensitivity of detection. However, it may also increase background. In most cases, a 2 hour incubation is sufficient for detection.)
7. Transfer membrane to a tray containing 20 ml TBST and wash for 5 minutes with gentle agitation. Repeat wash once more.
8. Transfer membrane to a tray containing the secondary antibody. Dilute the secondary antibody according to the manufacturer's recommendation into dilution buffer. Incubate with gentle agitation for 1 hour.
9. Wash two times in TBST as described in Step 7.
10. (Optional) Transfer blot from to a tray containing TBS and wash for 5 minutes to remove detergent.

Detection Reactions

If you are using a chemiluminescence kit, please refer to the manufacturer's instructions. If you wish to use alkaline phosphatase to detect your protein, the protocol on the next page is provided for your convenience. Other detection systems may be used. Please refer to Harlow and Lane, 1988 for a discussion of these systems.
1. Prepare fresh substrate solution immediately before use.  
   (For alkaline phosphatase conjugated antibody, add 66 µl of the NBT stock to 10 ml alkaline phosphatase buffer and mix well. Then add 33 µl of the BCIP stock and mix thoroughly. Use within 1 hour.)

2. Rinse the blot twice with enough alkaline phosphatase buffer to cover, then add 10 ml of the substrate solution. Incubate with gentle agitation at room temperature and watch for color development.  
   (When detecting higher concentrations of protein, the purple signal should develop within 10 minutes. Lower concentrations will take longer to develop but should be visible within 30 minutes. Color development will continue for up to 4 hours; however, high backgrounds will occur with longer incubation times. To decrease background, cover tray with aluminum foil.)

3. Stop the color development by washing the membrane in distilled water for 10 minutes. Change the water at least once during the 10 minute incubation.

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Technical Service, continued

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3E Company
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References


- For Immunoprecipitation, see Chapter 11, pp. 421-470.
- For Immunoblotting (Westerns), see Chapter 12, pp. 471-510.
- For Immunoassays (ELISA), see Chapter 14, pp. 553-612.