Alkaline Phosphatase (AP)-conjugated Antibodies

Catalog nos. R932-35, R942-25, R952-25, R962-25

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Important Information

Types of Antibodies

This manual is supplied with the alkaline phosphatase (AP)-conjugated antibodies listed below.

Antibody	Catalog no.
Anti-His(C-term)-AP Antibody	R932-25
Anti-HisG-AP Antibody	R942-25
Anti-*myc*-AP Antibody	R952-25
Anti-V5-AP Antibody	R962-25

Shipping/Storage

Each AP-conjugated antibody is shipped and stored at +4°C. Each product is guaranteed for six months from the date of receipt if properly stored.

For long-term storage, aliquot the antibody and store at -20°C or -80°C. Repeated freezing and thawing is not recommended as it may result in loss of antibody activity.

Contents

Each AP-conjugated mouse monoclonal antibody is supplied in Phosphate-Buffered Saline (PBS) and 0.01% sodium azide (added as a preservative). The table below provides information on the concentration of antibody, amount supplied, and the antibody subtype for each AP-conjugated antibody. The concentration of antibody provided is generally in the range of 1-2 mg/ml. The amount of antibody provided is sufficient for 25 western blots at a 10 ml working solution.

Antibody	Concentration	Amount	Subtype
Anti-HisG-AP	refer to label on tube	125 µl (25 westerns)†	IgG_{2a}
Anti-His(C-term)-AP	refer to label on tube	125 µl (25 westerns)†	IgG_{2b}
Anti-*myc*-AP	refer to label on tube	125 µl (25 westerns)†	IgG_1
Anti-V5-AP	refer to label on tube	125 µl (25 westerns)†	IgG_{2a}

†Assumes 10 ml buffer per western blot.

Antibody Conjugation

Each AP-conjugated antibody was prepared by crosslinking the appropriate primary antibody with alkaline phosphatase using glutaraldehyde (Harlow and Lane, 1988).

Product Specifications

For each AP-conjugated antibody, western blot experiments with 100 ng of recombinant Positope™ control protein gave a detectable signal within 15 minutes using a chromogenic substrate following the protocol on pages 3-4. The Positope™ control protein is a 53 kDa recombinant protein that contains seven epitope tags, including His(C-term), HisG, *c-myc*, and V5.

Methods

Overview

Introduction

The Anti-His(C-term)-AP, Anti-HisG-AP, Anti-*myc*-AP, and Anti-V5-AP antibodies allow detection of recombinant fusions proteins expressed from vectors containing the His(C-term), HisG, *c-myc*, and V5 epitopes, respectively. Most of the expression vectors available from Invitrogen contain one or more of these epitope tags. The antibodies may also be used to detect recombinant proteins expressed from vectors sold by other manufacturers (if they contain the appropriate epitope tag). For more information about the various expression vectors available from Invitrogen, please refer to our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 5).

Epitope

The table below describes the epitope recognized by each AP-conjugated antibody.

Product	Epitope	Amino Acid Sequence of Epitope
Anti-His(C-term)-AP Antibody	Detects fusion proteins containing a C-terminal polyhistidine (6xHis) tag (requires the free carboxyl group for detection (Lindner *et al.*, 1997)	His-His-His-His-His-His-COOH
Anti-HisG-AP Antibody	Detects fusion proteins containing a polyhistidine (6xHis) tag followed by glycine	His-His-His-His-His-His-Gly
Anti-*myc*-AP Antibody	Detects fusion proteins containing a 10 amino acid epitope derived from *c-myc* (Evan *et al.*, 1985)	Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu
Anti-V5-AP Antibody	Detects fusion proteins containing a 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991)	Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr

Antibody Specificity

All AP-conjugated antibodies have been tested in immunoblotting procedures. Low background was observed using the protocol on pages 3-4. No crossreactivity has been observed in bacterial lysates.

Important

Do not dilute the AP-conjugated antibody into PBS. Phosphate inhibits alkaline phosphatase. Use Tris-Buffered Saline (TBS) instead (see below).

Recommended Dilutions

For western blots, we recommend diluting the AP-conjugated antibody 1:2000 into Tris-Buffered Saline (TBS) containing 0.1% (v/v) Tween-20 and 1% (w/v) nonfat dry milk.

If you use a different buffer for washing and blocking your blots, then dilute as described above with that buffer. You may use other blocking agents such as bovine serum albumin (BSA) or gelatin.

Western Blotting (Immunoblotting)

Introduction

Many western blot procedures can be used for detection of recombinant fusion proteins with the appropriate AP-conjugated antibody. The following general protocol is included for your convenience. Other protocols and detection reagents are suitable. For details and other protocols, please refer to published references (Ausubel *et al.*, 1994; Harlow and Lane, 1988). The table below outlines the basic steps of a western blot.

Step	Description
1	Run an SDS polyacrylamide gel of the purified or partially purified protein or cell lysate with appropriate controls.
2	Transfer the proteins electrophoretically to a nitrocellulose, PVDF, or nylon membrane.
3	Probe the blot with the appropriate AP-conjugated antibody.
4	Detect fusion protein using the appropriate detection reagents.

Solutions Required

The following materials and solutions are needed for immunoblotting:

- Phosphate-Buffered Saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4, pH 7.3)
- Tris-Buffered Saline (TBS: 20 mM Tris-HCl, 140 mM NaCl, pH 7.5)
- Tris-Buffered Saline + Tween 20 (TBST: TBS plus 0.1% Tween-20, v/v)
- Blocking buffer (PBS + 5% nonfat dry milk, w/v)
- Dilution buffer (TBST + 1% nonfat dry milk, w/v)
- Appropriate AP-conjugated Antibody
- Enzyme Substrates for alkaline phosphatase reaction: 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 $MgCl_2$, pH 9.5)

Polyacrylamide Gel Electrophoresis

To facilitate separation of your recombinant protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Invitrogen. The patented NuPAGE® Gel System avoids the protein modifications associated with Laemmli-type SDS-PAGE, ensuring optimal separation for protein analysis. In addition, Invitrogen also carries a large selection of molecular weight protein standards and staining kits for visualization of proteins. For more information about the appropriate gels, standards, and stains to use to visualize your recombinant protein, please refer to our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 5).

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Immunoblotting Protocol

Prepare an SDS polyacrylamide gel (either Tris/Glycine or Tris/Tricine) designed to resolve your recombinant protein or purchase Novex® pre-cast gels (see the previous page). Prepare your samples for electrophoresis. For information about SDS-polyacrylamide gel electrophoresis, please see Ausubel *et al.*, 1994. Remember to load at least 100 ng of your purified recombinant protein onto the gel to get a strong signal.

1. Load your samples and electrophorese your SDS polyacrylamide gel.
2. Transfer proteins to nitrocellulose or any other suitable membrane electrophoretically. using 25 mM Tris, pH 8.3, 192 mM glycine, 20% (v/v) methanol as a transfer buffer. Other transfer buffers are suitable.

   Note: If you are using NuPAGE® gels or other types of pre-cast gels, other transfer buffers may be required. Please refer to the manufacturer's instructions to transfer proteins to the membrane of choice.
3. Run at 100V, 150 mA (100V, 240 mA at the finish) for 1 hour. Be sure to have a cooling system in place. You may also transfer overnight at 30V, 40 mA (which will be 30V, 90 mA at the finish).
4. Remove membrane and incubate it in 10 ml blocking solution. Gently agitate using a rocker platform for 1 hour at room temperature.

   Note: You can store the blot overnight at this step if needed. Keep the blot in blocking solution and store at +4°C covered with plastic wrap.
5. Wash membrane in 20 ml TBST for 5 minutes with gentle agitation. Repeat twice.
6. Transfer membrane to a tray containing the appropriate AP-conjugated antibody diluted 1:2000 in 10 ml dilution buffer (2 µl of AP-conjugated antibody diluted into 10 ml dilution buffer). Incubate with gentle agitation for at least 2 hours at room temperature.

   (Overnight incubation may be preferred since longer incubations may increase sensitivity of detection. However, longer incubations will 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 three times more.
8. Proceed to **Alkaline Phosphatase Detection Reaction**, next page.

continued on next page

Alkaline Phosphatase Detection Reaction

A protocol is provided below for detection of alkaline phosphatase activity using a colorimetric substrate. Chemiluminescence substrates may also be used for detection using enhanced chemiluminescence. Please follow the manufacturer's instructions to perform the detection reaction.

1. Transfer membrane from Step 7, previous page, to a tray containing TBS and wash for 5 minutes to remove detergent.
2. Prepare fresh substrate solution immediately before use (see page 2).

   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.
3. Rinse the blot twice with alkaline phosphatase buffer, then add 10 ml of the substrate solution. Incubate with gentle agitation at room temperature and watch for color development. Proceed to the next step

   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, higher backgrounds will occur with longer incubation times.
4. 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.
5. Air-dry membrane on filter paper.

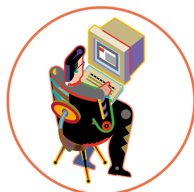
Troubleshooting

The table below lists some potential problems and possible solutions that you may use to help you troubleshoot your western blotting.

Problem	Reason	Solution
No signal	Poor or no transfer	Stain membrane with Ponceau S to check degree of transfer, then re-run the gel and repeat transfer
	Antibody too dilute	Use more antibody
	Protein too dilute	Load more protein
	Old detection reagents	Prepare fresh detection reagents immediately before use
High background	Antibody too concentrated	Titrate the antibody and use the maximal dilution that gives a detectable signal in a reasonable amount of time
	Insufficient blocking	Increase incubation time in blocking solution Include Tween-20, BSA, or other blocking agents in the blocking and washing solutions
Multiple protein bands	Proteolysis of the protein	Use protease inhibitors when preparing cell lysates
	Inefficient reduction of the protein	Resuspend samples in SDS-PAGE sample buffer containing fresh reducing agent and boil the samples for 5 minutes prior to electrophoresing your gel

Technical Service

World Wide Web



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http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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1. On the home page, go to the left-hand column under ‘Technical Resources’ and select ‘MSDS Requests’.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the ‘Add Another’ button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the ‘Submit’ button. Your MSDS will be sent within 24 hours.

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Emergency Information

In the event of an emergency, customers of Invitrogen can call the 3E Company, 24 hours a day, 7 days a week for disposal or spill information. The 3E Company can also connect the customer with poison control or with the University of California at San Diego Medical Center doctors.

3E Company
Voice: 1-760-602-8700

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