Anti-Thio™ Antibody

Catalog no. R920-25

Version D
010302
28-0098

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Important Information

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Fifty microliters of the Anti-Thio™ mouse monoclonal $IgG_{1\kappa}$ antibody is isolated from mouse ascites fluid and Protein A affinity purified. It is supplied in phosphate-buffered saline (PBS) with 0.05% sodium azide added as a preservative and 1 mg/ml bovine serum albumin (BSA) for stability. Enough reagent is provided to perform 25 Western blots.

Storage and Shipping Conditions

The Anti-Thio™ Antibody is shipped and stored at +4°C. 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.

Product Qualification

The Anti-Thio™ Antibody is functionally tested by Western blot using protocols described in the manual and by ELISA. The antibody must react specifically with 100 ng of a thioredoxin-chloramphenicol acetyl transferase (CAT) fusion protein expressed from the vector pTrxFus. Western blots must reveal a strong signal, with no non-specific *E. coli* background, after 10 minutes of color development.

Introduction

Overview

Introduction

The Anti-Thio™ antibody is a monoclonal antibody that allows detection of thioredoxin and thioredoxin fusion proteins expressed from the pBAD/Thio-TOPO® and pBAD-DEST49 vectors (Catalog nos. K370-01 and 12283-016, respectively). Genes cloned into the multiple cloning sites of these vectors are expressed as C-terminal fusions to *E. coli* thioredoxin (N-terminus of your protein is fused to the C-terminus of thioredoxin). The Anti-Thio™ antibody recognizes the thioredoxin protein and allows detection in expression and purification experiments.

Note that the Anti-Thio™ antibody will recognize recombinant thioredoxin proteins with peptides inserted at the active site (internal peptide fusions) as well as His-patch (HP) thioredoxin from the pBAD/Thio-TOPO® and pBAD-DEST49 vectors. See LaVallie, *et al.*, 1993 for more information about internal peptide fusions.

Specificity of the Antibody

The Anti-Thio™ antibody has been tested in both immunoblotting and ELISA procedures. In Western blot experiments with purified protein, 100 ng of recombinant protein gave a good signal after a 60 minute incubation with the Anti-Thio™ antibody, a 60 minute incubation with alkaline phosphatase-conjugated anti-mouse secondary antibody, and a 10 minute incubation for color development.

Recommended Dilutions

We recommend the following dilutions of the solution of antibody for these applications:

- For Western blots, dilute 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.
- For ELISA assays, dilute 1:500 in TBS or PBS with 1% gelatin. Note: A background level of 1-7.5 % is detected with ELISA assays due to endogenous *E. coli* thioredoxin.

If you use a different buffer for washing and blocking your blots, then dilute as described above. 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-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.

Western and Dot Blot

Introduction

This procedure can be used for detection of fusion protein expression particularly when levels of expression are low. The table below outlines the basic steps of a Western blot. For information on immunoprecipitaion, immunoblotting and immunoassays, refer to *Antibodies: A Laboratory Manual* (Harlow and Lane, 1988).

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 nylon or nitrocellulose membrane
3	Probe the blot with Anti-Thio™ antibody
4	Incubate the blot with anti-mouse IgG secondary antibody conjugated to a color development enzyme such as alkaline phosphatase or horseradish peroxidase (HRP)
5	Add the color development substrate and watch for the appearance of colored band on the nitrocellulose

Suggested Solutions

We use alkaline phosphatase conjugated to anti-mouse IgG antibody in order to detect binding of the Anti-Thio™ antibody to the recombinant protein. Other detection methods can be used to detect your protein. Note that alkaline phosphatase is more sensitive than HRP. The following materials and solutions are needed for immunoblotting:

- 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)
- Secondary Antibody: Anti-Mouse IgG (whole molecule) Alkaline Phosphatase (Sigma-Aldrich, Catalog no. A3562)
- 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. Store at -20°C and wrap in foil to protect from light.
- Alkaline phosphatase buffer (100 mM diethanolamine, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5)

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Western and Dot 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, see Ausubel, *et al.*, 1994.) Remember that you need to load at least 100 ng of your recombinant protein onto the gel in order to get a good signal.

1. Load your samples and electrophorese your SDS polyacrylamide gel.
2. Transfer proteins to nitrocellulose electrophoretically. We use 25 mM Tris, 192 mM glycine, and 20% v/v methanol, pH 8.3 as a transfer buffer.
3. Run at 100V, 150 mA (100V, 240 mA at the finish) for 1 hour. Be sure to have a cooling system in place and operational with these electrophoretic settings. You may also transfer overnight at 30V, 40 mA (30V, 90 mA at the finish).
4. Remove nitrocellulose and incubate it in 10 ml blocking solution. Gently agitate using a rocker platform for 1 hour at room temperature.
5. Wash nitrocellulose in 20 ml TBST for 5 minutes with gentle agitation. Repeat wash once more.
6. Transfer membrane to a tray containing Anti-Thio™ antibody diluted 1:5000 in 10 ml dilution buffer (2 µl of Anti-Thio™ antibody diluted into 10 ml dilution buffer). Incubate with gentle agitation for 1-2 hours.

   (Overnight incubation may be preferred. Longer incubations may increase sensitivity of detection. In our hands, a 1 hour incubation is sufficient for detection of most thioredoxin fusion proteins.)
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. Proceed to **Detection Reaction**.

Detection Reaction

The protocol below was developed using alkaline phosphatase-conjugated secondary antibody. If using a different system, be sure to follow the manufacturer's instructions.

1. Transfer blot from Step 9, above, to a tray containing TBS and wash for 5 minutes to remove detergent.
2. 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.)
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.

   (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.)
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.

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Western and Dot Blot, continued

Dot Blot Protocol

This protocol is used to quickly detect the presence of recombinant protein. This method can be used to screen a variety of clones in order to find the highest expressing clone. Be sure to spot equivalent amounts of protein for each sample.

1. Make serial dilutions of samples (purified or partially purified protein or cell lysates) in 10 mM Tris-HCl, 25 mM EDTA, pH 8.0. The lowest dilution should have at least 100 ng of protein present.
2. Spot 1 µl of each sample onto nitrocellulose paper, or alternatively use a slot blot apparatus.
3. Allow membrane to air-dry.
4. Proceed with **Immunoblotting Protocol**, steps 4-9, then the **Detection Reaction**, steps 1-5 on page 3.

Important

Do not use "shockates" in a dot blot assay as the epitope recognized by the Anti-Thio™ antibody is masked.

Troubleshooting

We have observed, in some instances, that the thioredoxin domain is not recognized in Western blots as strongly in some fusions as in others. Increasing the length of the blocking step from 1 hour to overnight can increase the level of detection. A longer incubation with the Anti-Thio™ antibody or increasing the amount of antibody used will increase the signal; however, these two steps may also increase background in a Western blot.

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Technical Service, continued

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References

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). Current Protocols in Molecular Biology (New York: Greene Publishing Associates and Wiley-Interscience).

Harlow, E., and Lane, D. (1988). Antibodies: A Laboratory Manual (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory).

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.

LaVallie, E. R., DiBlasio, E. A., Kovacic, S., Grant, K. L., Schendel, P. F., and McCoy, J. M. (1993). A Thioredoxin Gene Fusion Expression System That Circumvents Inclusion Body Formation in the *E. coli* Cytoplasm. Bio/Technology *11*, 187-193.