Anti-HisG Antibody
Anti-HisG-HRP Antibody

Catalog nos. R940-25 and R941-25

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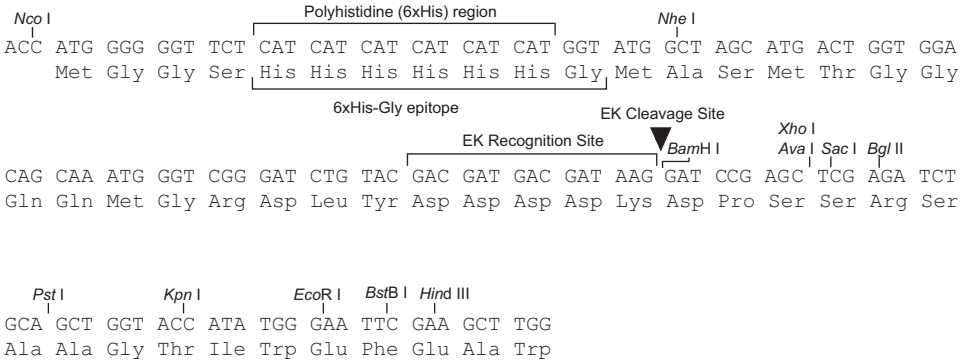
Overview

Introduction

The Anti-HisG and Anti-HisG-HRP antibodies allow detection of recombinant proteins containing a polyhistidine sequence. The Anti-HisG antibodies recognize the sequence -His-His-His-His-His-His-Gly (6xHis-Gly epitope). The antibodies can be used to detect expression of fusion proteins from bacterial, insect, and mammalian cells.

Anti-HisG Epitope

The diagram below shows the location of the epitope recognized by the Anti-HisG antibodies. The example shown is the leader peptide from the pTrcHis B vector. The leader peptide is the same in all of the Invitrogen vectors up to and including the aspartic acid following the lysine in the enterokinase recognition site. The multiple cloning site varies between vectors.



Contents

The table below provides information on the concentration of antibody, buffer, and amount supplied.

The Anti-HisG Antibody is a mouse monoclonal IgG_{2a} antibody. Anti-HisG-HRP Antibody was prepared by crosslinking the Anti-HisG Antibody with horseradish peroxidase using glutaraldehyde.

Antibody	Concentration	Buffer	Amount
Anti-HisG	Refer to label on tube	PBS, 0.01% azide	50 µl (25 westerns)†
Anti-HisG-HRP	Refer to label on tube	PBS	50 µl (25 westerns)†

†Assumes 10 ml buffer per western blot.

continued on next page

Overview, Continued

Shipping/Storage

The Anti-HisG Antibodies are shipped and stored at +4°C. Each product is guaranteed for six months from the date of receipt.

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.

Antibody Specificity

Both antibodies have been tested in immunoblotting and ELISA procedures. Low background was observed using chemiluminescent or alkaline phosphatase reagents for detection.

In western blot experiments with purified protein, 50 ng (for Anti-HisG-HRP Antibody) or 100 ng (for Anti-HisG Antibody) of recombinant Positope™ protein gave a detectable signal using the protocol on page 4.

Note: The Anti-HisG Antibody has also been used successfully to immunoprecipitate fusion proteins that contain the 6xHis tag.

Crossreactivity

Using chemiluminescence as the detection method, no crossreactivity has been observed in bacterial lysates. In mammalian lysates, a few crossreactive proteins have been observed upon overexposure of blots.

Recommended Dilutions

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

- For western blots, dilute 1:5000 into Phosphate-Buffered Saline (PBS) containing 0.05% Tween-20 and 5% nonfat, dry milk (PBSTM).
- For ELISA assays, serially dilute into PBSTM and test dilutions to determine the best dilution of antibody for your application.

You may use other buffers of your choice for dilution or blocking agents such as bovine serum albumin (BSA) or gelatin.

Important

If you use alkaline phosphatase-conjugated secondary antibody, do not use PBS. Phosphate inhibits alkaline phosphatase. Use Tris-Buffered Saline (TBS) instead.

Be sure to wash the western blot or microtiter wells before adding the horseradish peroxidase-conjugated secondary antibody. Azide will inhibit HRP activity.

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.

Step	Description
1	Run an SDS polyacrylamide gel of either the purified protein, partially purified protein or cell lysate with appropriate controls.
2	Transfer the proteins electrophoretically to a PVDF or nitrocellulose membrane.
3	Probe the blot with Anti-HisG Antibody or the Anti-HisG-HRP Antibody.
4	If you probe with the Anti-HisG Antibody, incubate the blot with anti-mouse IgG secondary antibody conjugated to an enzyme such as alkaline phosphatase or horseradish peroxidase (HRP).
5	Detect fusion protein using the appropriate detection method of choice.

Suggested Solutions

We use chemiluminescence to detect binding of the Anti-HisG Antibodies to the recombinant protein. Other detection methods can be used. The following materials and solutions are needed for immunoblotting and chemiluminescent detection:

- Phosphate-Buffered Saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4
- Phosphate-Buffered Saline + Tween 20 (PBST: PBS plus 0.05% Tween-20, v/v)
- Blocking buffer (PBST + 5% nonfat, dry milk, w/v)
- Secondary Antibody: Anti-Mouse IgG (whole molecule) HRP

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Immunoblotting Protocol

Prepare an SDS polyacrylamide gel designed to resolve your recombinant protein or you may purchase Novex® pre-cast gels (please call Technical Service for more information). For information about SDS-polyacrylamide gel electrophoresis, please see Ausubel *et al.*, 1994.

1. Prepare and load your samples for electrophoresis. Load at least 5 to 50 ng of your recombinant protein onto the gel in order to get a good signal.
2. Transfer proteins to PVDF or nitrocellulose membrane electrophoretically. We use 25 mM Tris (pH 8.3), 192 mM glycine, and 20% v/v methanol as a transfer buffer.
3. Transfer at 100 V, 150 mA (100 V, 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 30 V, 40 mA (30 V, 90 mA at the finish).
4. Remove the nitrocellulose membrane and place it in a clean tray containing 10 ml of blocking buffer. Gently agitate using a rocking platform for 1 hour at room temperature.
5. Wash the nitrocellulose membrane twice in 20 ml of PBST for 5 minutes with gentle agitation.
6. Transfer membrane to a tray containing either the Anti-HisG or the Anti-HisG-HRP Antibody diluted 1:5000 in 10 ml blocking buffer (2 µl of antibody diluted into 10 ml blocking buffer). Incubate with gentle agitation for 1-2 hours.

   Note: Overnight incubation may be preferred, since longer incubations may increase the sensitivity of detection.
7. Decant the antibody solution and wash the membrane twice with 20 ml of PBST for 5 minutes with gentle agitation. If you are using the Anti-HisG-HRP Antibody, proceed to detection.
8. If you are using the Anti-HisG Antibody, transfer membrane to a tray containing the secondary antibody. Dilute the secondary antibody according to the manufacturer's recommendation into blocking buffer. Incubate for 1 hour with gentle agitation.
9. Wash the membrane as described in Step 7.

Detection Reaction

We use enhanced chemiluminescence to detect the fusion proteins. Please follow the manufacturer's instructions. Other detection methods are suitable.

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 baculovirus or mammalian 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 30 ng of protein present. Nitrocellulose membrane can bind approximately 100 µg protein per cm^2.
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 to the **Immunoblotting Protocol**, steps 4-9, above, then to the **Detection Reaction**.

continued on next page

Western and Dot Blot, Continued

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

Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

In addition to western blots, the Anti-HisG Antibodies can be used in immunoassays. A sample protocol is provided for your convenience or you may use a protocol of your choice. For more information, please refer to *Antibodies* (Harlow and Lane, 1988) and *Current Protocols in Molecular Biology* (Ausubel *et al*., 1994), unit 11.2.

Guidelines

Please follow these guidelines when setting up your ELISA.

- Include controls for cross-reactivity and nonspecific binding to host cell proteins
- Be sure that all experimental conditions are kept constant to ensure reproducibility
- Determine optimal dilution of Anti-HisG or Anti-HisG-HRP Antibody for use with your antigen
- Always include a standard curve with each plate
- Analyze samples in duplicate
- Be sure that the concentration of antigen falls within the dynamic range of the standard curve.

Detection of Antigen

Using ELISA assay, you can detect 1 ng/ml to 1 µg/ml antigen in a bacterial cell lysate. Sandwich ELISA is more sensitive than direct ELISA.

Buffers

We have used the following buffers with these antibodies.

- PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM $NaHPO_4$, 1.8 mM KH_2PO_4
- PBST: PBS, 0.05% Tween-20
- PBSTM: PBST, 5% nonfat, dry milk

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General Procedure

The procedure below is an example of a direct ELISA to detect the amount of antigen in a bacterial cell lysate. For details, please refer to Unit 11.2 of *Current Protocols in Molecular Biology* (Ausubel *et al*., 1994). Please note that other protocols are suitable.

1. Prepare a 10 µg/ml solution of antigen in PBS. This will be used for your standard curve so it should be as pure as possible.
2. Take a polystyrene 96-well plate and serially dilute the antigen solution across the columns so that each row has a different dilution of antigen. Use PBSTM as the dilution buffer and 50 µl as the final volume in each well. Leave column 12 blank as a background control. Repeat with a second plate. **Note**: We recommend that you include controls such as a lysate that does not contain the antigen of interest.
3. Cover the plates and incubate overnight at +4°C or 2 hours at room temperature to allow antigen to bind to the plate.
4. Remove antigen solution and wash the wells three times with PBS.
5. Add 0.2 ml of PBSTM to each well and incubate the plates for 1 hour at room temperature.
6. Remove PBSTM and wash the plates three times with PBST.
7. Serially dilute Anti-HisG or Anti-HisG-HRP Antibody across the rows. Use PBSTM as the diluting buffer and 50 µl as the final volume in each well. Start with a 1:500 or 1:1000 dilution in row A.
8. Cover and incubate the plates at room temperature for 2 hours.
9. Remove antibody and wash wells three times with PBST. If you used Anti-HisG-HRP Antibody, proceed to detection reaction.
10. For unconjugated Anti-HisG Antibody, add 50 µl of diluted anti-mouse HRP-conjugated secondary antibody to each well. Use PBSTM as the dilution buffer. For the appropriate dilution of secondary antibody, please see the manufacturer's instructions.
11. Incubate the plates at room temperature for 30 to 60 minutes.
12. Remove the antibody solution and wash the wells four times with PBS.
13. Proceed to **ELISA Detection Reaction**, below.

ELISA Detection Reaction

The protocol uses a sensitive chromogenic substrate for detection of HRP-labeled reagents. For other detection methods, please refer to *Antibodies* (Harlow and Lane, 1988). This protocol makes enough substrate solution for up to two 96-well microtiter plates. Pre-made substrate solution is available from Sigma (Catalog no. T8865).

1. Dissolve 0.1 mg of TMB (3, 3′, 5, 5′-tetramethylbenzidine, Sigma Catalog no. T2885) in 0.1 ml of dimethylsulfoxide (DMSO).
2. Add 9.9 ml of 0.1 M sodium acetate, pH 6.0.
3. Filter through Whatman No. 1 paper or equivalent.
4. Add hydrogen peroxide to a final concentration of 0.01%.
5. Add 50 µl of the substrate solution to each well.
6. Incubate the plates for 10-30 minutes at room temperature. Positives appear pale blue.
7. Add 50 µl of 1 M H_2SO_4 to each well. Positives now appear bright yellow.
8. Read the results at 450 nm using a spectrophotometer.

continued on next page

Analysis of Experiment

Plot absorbance versus known antigen concentration on semilog paper to analyze each antibody dilution. For a working dilution of the antibody, choose the dilution that provides maximum sensitivity over a linear range of antigen concentrations and a minimum background.

Analyzing Lysates

Once you have identified the optimal working dilution of Anti-HisG or Anti-HisG-HRP, you are ready to analyze your lysates.

1. Prepare a ~10 µg/ml solution of your lysate in PBS.
2. Take a polystyrene 96-well plate and serially dilute the lysates across the columns so that each row has a different dilution of antigen. Use PBSTM as the diluting buffer and 50 µl as the final volume in each well. Leave column 12 blank as a background control. Repeat with a second plate.
3. Cover the plates and incubate overnight at 4°C or 2 hours at room temperature.
4. Remove antigen solution and wash with wells with PBS three times.
5. Add 0.2 ml of PBSTM to each well and incubate for 1 hour at room temperature.
6. Remove PBSTM and wash the wells three times with PBST
7. Add 50 µl of the appropriate dilution of Anti-HisG or Anti-HisG-HRP Antibody in PBSTM.
8. Cover and incubate the plates at room temperature for 2 hours.
9. Remove antibody and wash wells three times with PBST. If you used Anti-HisG-HRP Antibody, proceed to detection reaction.
10. For Anti-HisG Antibody, add 50 µl of anti-mouse HRP-conjugated secondary antibody diluted in PBSTM to each well. For the appropriate dilution of secondary antibody, please see the manufacturer's instructions.
11. Incubate the plates for 30-60 minutes at room temperature.
12. Remove antibody and wash wells four times with PBS.
13. Proceed to **ELISA Detection Reaction**, previous page.

Immunoprecipitation

Immuno-precipitation Protocol

The Anti-HisG and Anti-HisG-HRP Antibodies can be used to immunoprecipitate fusion proteins that contain the -His-His-His-His-His-His-Gly epitope. The procedure below describes a general immunoprecipitation protocol. For more details, please refer to *Antibodies* (Harlow and Lane, 1988).

1. Prepare a lysis buffer of your choice. We recommend using either:
   - RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris, pH 7.5) or
   - NP-40 lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris, pH 8.0)

   When preparing the lysis buffer, you may want to include a cocktail of protease inhibitors such as 10-100 µM leupeptin, 1-10 mM EDTA, 1 µM pepstatin, and 0.2-1 mM PMSF (from 100 mM stock in 2-propanol).
2. Prepare cell lysate using the appropriate lysis buffer. For mammalian and insect cells, use approximately 5 x 10^6 to 1 x 10^7 cells/ml of lysis buffer. For

   E. coli and yeast cells, use approximately 1 x 10^9 cells/ml of lysis buffer.
3. Centrifuge the lysate for 20 minutes at 10,000 x g at +4°C.
4. Carefully transfer the supernatant to a sterile microcentrifuge tube and place on ice.
5. Add 50 µl of Protein-G Sepharose® resin slurry (50% slurry in lysis buffer) per 1 ml of supernatant to pre-clear the lysate.
6. Rock at +4°C for 1 hour.
7. Centrifuge for 1 minute at 10,000 x g at +4°C.
8. Transfer supernatant to a sterile microcentrifuge tube and place on ice.
9. Add 1-2 µg (typically, 1-2 µl) of the Anti-HisG or Anti-HisG-HRP Antibodies and 50 µl of the Protein-G Sepharose® resin slurry to the supernatant. Incubate the tube for 2-24 hours at +4°C with gentle rocking on a rocking platform.
10. Centrifuge for 1 minute at 10,000 x g at +4°C.
11. Remove the supernatant.
12. Wash the resin twice with 500 µl of lysis buffer.
13. The fusion protein immune complexes may now be used in the appropriate assay.
14. For SDS polyacrylamide gels, add 50 µl of SDS-PAGE sample buffer (2% SDS, 10% glycerol, 100 mM DTT, 60 mM Tris pH 6.8, and 0.001% bromophenol blue) to the resin. Heat the sample at 85°C for 2 minutes. Centrifuge for 1 minute at 10,000 x g and load the supernatant onto an appropriate gel.

Technical Service

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...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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Technical Service, Continued

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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Product Qualification

Product Qualification

Both antibodies are functionally tested by western blot using the protocols on page 4.

Anti-HisG Antibody

The antibody must react specifically with 100 ng of an *E. coli* expressed fusion protein containing a 6xHis-Gly epitope. Western blots must reveal a strong signal, with no non-specific background, after 10 minutes of color development.

Anti-HisG-HRP Antibody

The antibody must react specifically with 50 ng of an *E. coli* expressed fusion protein containing a 6xHis-Gly epitope. Western blots must reveal a strong signal, with no non-specific background, after development with a chemiluminescent substrate followed by a 1 minute exposure to x-ray film.

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.* Greene Publishing Associates and Wiley-Interscience, New York.

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

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.

Lindner, P., Bauer, K. Krebber, A., Nieba, L., Kremmer, E., Krebber, C., Honegger, A., Klinger, B., Mocikat, R., and Plueckthun, A. (1997) Specific Detection of His-tagged Proteins With Recombinant Anti-His Tag scFv-phosphatase or scFv-phage Fusions. *Biotechniques* **22**:140-149.