Anti-V5 Antibody
Anti-V5-HRP Antibody

Catalog nos. R960-25, R961-25

Version F
073001
28-0140
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Overview

Introduction

The Anti-V5 Antibody allows detection of recombinant proteins containing the V5 epitope. This epitope is found in the P and V proteins of the paramyxovirus, SV5 (Southern, et al., 1991). The Anti-V5 Antibody recognizes the 14 amino acid sequence:

\[
\text{Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr}
\]

Contents

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

The Anti-V5 Antibody is a mouse monoclonal IgG2a antibody. Anti-V5-HRP Antibody was prepared by crosslinking the Anti-V5 Antibody with horseradish peroxidase using glutaraldehyde.

For instructions on how to obtain safety information about sodium azide, please refer to the MSDS Information section (see page 12).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Concentration</th>
<th>Buffer</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-V5</td>
<td>refer to label on tube</td>
<td>PBS, 0.01% azide</td>
<td>50 µl (25 westerns)†</td>
</tr>
<tr>
<td>Anti-V5-HRP</td>
<td>refer to label on tube</td>
<td>PBS</td>
<td>50 µl (25 westerns)†</td>
</tr>
</tbody>
</table>

†Assumes 10 ml buffer per western blot.

Shipping/Storage

The Anti-V5 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, 25 ng (for Anti-V5 Antibody) or 50 ng (for Anti-V5-HRP Antibody) of recombinant Positope™ protein (Catalog no. R900-50) gave a detectable signal using the immunoblotting protocol on page 4.

Note: The Anti-V5 Antibody has also been used successfully to immunoprecipitate fusion proteins that contain the V5 epitope.

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.

continued on next page
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) or Tris-Buffered Saline (TBS) containing 0.05% Tween-20 and 5% nonfat, dry milk (PBSTM or TBSTM).

- For ELISA assays, serially dilute into PBSTM or TBSTM and test dilutions to determine the best dilution of antibody for your application.

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.

Important

If you use azide in your HRP-conjugated antibody dilution buffers, be sure to wash the western blot or microtiter wells thoroughly before adding the color development solution. Azide will inhibit horseradish peroxidase activity.

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

Product Qualification

The Anti-V5 Antibody and the Anti-V5-HRP Antibody are functionally tested by western blot using the protocols described in the manual.

**Anti-V5 Antibody**

The antibody must react specifically with 20 ng of an *E. coli* expressed fusion protein containing a V5 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.

**Anti-V5-HRP Antibody**

The antibody must react specifically with 500 ng of an *E. coli* expressed fusion protein containing a V5 epitope. Western blots must reveal a strong signal, with no non-specific background, after development with a chemiluminescent substrate followed by a 10 minute exposure to x-ray film.
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.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Run an SDS polyacrylamide gel of the purified or partially purified protein or cell lysate with appropriate controls.</td>
</tr>
<tr>
<td>2</td>
<td>Transfer the proteins electrophoretically to a nylon or nitrocellulose membrane.</td>
</tr>
<tr>
<td>3</td>
<td>Probe the blot with Anti-V5 Antibody or the Anti-V5-HRP Antibody.</td>
</tr>
<tr>
<td>4</td>
<td>If you probe with the Anti-V5 Antibody, incubate the blot with anti-mouse IgG secondary antibody conjugated to an enzyme such as alkaline phosphatase or horseradish peroxidase (HRP).</td>
</tr>
<tr>
<td>5</td>
<td>Visualize fusion protein using the appropriate method.</td>
</tr>
</tbody>
</table>

Suggested Solutions

We use chemiluminescence to detect binding of the Anti-V5 Antibodies to the recombinant protein. Other detection methods can be used. The following items are needed for immunoblotting and chemiluminescent detection:

- Phosphate-Buffered Saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na$_2$HPO$_4$, 1.8 mM KH$_2$PO$_4$, pH 7.4)
- Tris-Buffered Saline (137 mM NaCl, 10 mM Tris, pH 8.0)
- 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 (Anti-V5 Antibody only)

Colorimetric detection methods can also be used to detect your protein. Substrates for colorimetric detection are described below. A detection protocol using chloronapthol is provided on the next page. Protocols using the other reagents can be found in Harlow and Lane, 1988, pages 507-509.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloronapthol</td>
<td>Intense blue-black product</td>
<td>Relatively insensitive\nControlled reaction with low background\nReadily photographed</td>
</tr>
<tr>
<td>Aminoethylcarbazole</td>
<td>Red reaction product\nMore sensitive</td>
<td>Harder to photograph</td>
</tr>
<tr>
<td>Diaminobenzidine</td>
<td>Brown reaction product can be enhanced with metals to form black product\nMost sensitive</td>
<td>Easy to overdevelop\nHigh background if overdeveloped</td>
</tr>
</tbody>
</table>
Western and Dot Blot, continued

Immunoblotting Protocol

Either prepare an SDS polyacrylamide gel designed to resolve your recombinant protein or 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, pages 10.2A.1-10.2A.9.

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 nitrocellulose membrane electrophoretically. We use 25 mM Tris (pH 8.3), 192 mM glycine, and 20% v/v methanol as a transfer buffer.

3. Run 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 incubate it in 10 ml blocking buffer. Gently agitate using a rocker platform for 1 hour at room temperature.

5. Wash the nitrocellulose membrane in 20 ml PBST 2X for 5 minutes each with gentle agitation.

6. Transfer membrane to a tray containing either the Anti-V5 or the Anti-V5-HRP Antibody diluted 1:5000 in 10 ml blocking buffer (2 µl of antibody diluted into 10 ml blocking buffer). Incubate at room temperature with gentle agitation for 1-2 hours. Generally, a 1 hour incubation is sufficient for detection. Overnight incubation may be preferred, since longer incubations may increase the sensitivity of detection. Overnight incubations should be performed at +4°C.

7. Transfer membrane to a tray containing 20 ml PBST and wash for 2 x 5 minutes with gentle agitation. If you are using the Anti-V5-HRP Antibody, proceed to detection, below.

8. If you are using the Anti-V5 Antibody, transfer membrane to a clean tray. Add secondary antibody diluted according to the manufacturer’s recommendation into blocking buffer. Incubate with gentle agitation for 1 hour.

9. Wash for 2 x 5 minutes as described in Step 7. Detect using the method of choice for your secondary antibody.

Detection Method

We use enhanced chemiluminescence to detect the fusion proteins. Using your system of choice, follow the manufacturer’s instructions to detect your protein.

If you are using chloronapthol, follow the protocol below. Protocols for aminoethylcarbazole and diaminobenzidine can be found in Harlow and Lane, 1988, pages 507-509.

continued on next page
Western and Dot Blot, continued

Detection Reaction

To detect using chloronapthol, prepare the following solutions:

- 3% chloronapthol (0.3 g chloronapthol in 10 ml absolute ethanol; store at -20°C)
- 50 mM Tris-HCl, pH 7.6
- 30% H₂O₂ (store at +4°C)

1. Prepare fresh substrate solution immediately before use. Add 0.1 ml of 3% chloronapthol to 10 ml of 50 mM Tris-HCl, pH 7.6. A white precipitate will form.
2. Remove the precipitate by filtering through Whatman No.1 filter paper (or similar).
3. Add 10 µl of 30% H₂O₂ to the buffered chloronapthol solution.
4. Add solution from Step 3 to washed blot and agitate at room temperature. Develop the blot until the bands are visible and dark enough for photography. Typical incubations should last no longer than 30 minutes.
5. To stop the reaction, remove the substrate solution and rinse with TBS.
6. Air-dry the membrane on filter paper.

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 cm².
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, previous page, then to the Detection Reaction.
Enzyme-Linked Immunosorbent Assay (ELISA)

**Introduction**

In addition to western blots, the Anti-V5 Antibodies can also be used in immunoassays. A sample protocol is provided for your convenience. Other protocols are suitable. 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 consider the items below 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-V5 or Anti-V5-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**

ELISAs can be used to detect 1 ng/ml to 1 µg/ml antigen in a bacterial cell lysate. Sandwich ELISAs are more sensitive than direct ELISAs.

**Buffers**

We have used the following buffers with these antibodies.

- PBS: 137 mM NaCl, 2.7 mM KCl, 10 mM NaHPO₄, 1.8 mM KH₂PO₄
- PBST: PBS, 0.05% Tween-20
- PBSTM: PBST, 5% nonfat, dry milk

*continued on next page*
ELISA, continued

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 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 including 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 with PBS three times.
5. Add 0.2 ml PBSTM to each well and incubate for 1 hour at room temperature.
6. Remove PBSTM and wash with PBST three times.
7. Serially dilute Anti-V5 or Anti-V5-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 plates at room temperature for 2 hours.
9. Remove antibody and wash wells three times with PBST. If you used Anti-V5-HRP Antibody, proceed to detection reaction.
10. For unconjugated Anti-V5 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 at room temperature for 30 to 60 minutes.
12. Remove antibody and wash wells four times with PBS. Proceed to ELISA Detection Reaction, below.

ELISA Detection Reaction

The protocol uses a sensitive chromogenic substrate for detection of HRP-labeled reagents. Other detection reagents are suitable. 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 other vendors (i.e. 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.
7. Add 50 µl of 1 M H₂SO₄ to each well. Positives now appear bright yellow.
8. Read the results at 450 nm using a spectrophotometer.
ELISA, continued

**Analysis of Experiment**

Plot absorbance versus known antigen concentration on semilog paper to analyze each antibody dilution. For a working dilution of antibody, choose the dilution that provides the maximum sensitivity over a linear range of antigen concentrations and a minimum binding (< 0.05 absorbance units) for background.

**Analyzing Lysates**

Once you have identified the optimal working dilution of Anti-V5 or Anti-V5-HRP, you are ready to analyze your lysates.

1. Prepare an ~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 PBSTM to each well and incubate for 1 hour at room temperature.
6. Remove PBSTM and wash with PBST three times
7. Add 50 µl of the appropriate dilution of Anti-V5 or Anti-V5-HRP Antibody in PBSTM.
8. Cover and incubate plates at room temperature for 2 hours.
9. Remove antibody and wash wells three times with PBST. If you used Anti-V5-HRP Antibody, proceed to detection reaction.
10. For Anti-V5 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 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

Immunoprecipitation Protocol

The Anti-V5 and Anti-V5-HRP Antibodies can be used to immunoprecipitate fusion proteins that contain the V5 epitope. The procedure below describes a general immunoprecipitation protocol; other protocols are suitable. For more details, please refer to Antibodies (Harlow and Lane, 1988).

1. Before beginning, you will need to prepare the appropriate lysis buffer. Many lysis buffers are suitable. We recommend using either:
   - RIPA buffer (150 mM NaCl, 1% NP-40, 0.5% deoxycholate (DOC), 0.1% SDS, 50 mM Tris, pH 7.5) OR
   - NP-40 or Triton-X-100 lysis buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 1% either NP-40 or Triton-X-100)

   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. Lyse cells using one of the lysis buffers above or the one of your choice. 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 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-V5 or Anti-V5-HRP Antibodies and 50 µl of the Protein-G Sepharose® resin slurry to the supernatant. Rock for 2-24 hours at +4°C.

10. Centrifuge for 1 minute at 10,000 x g at +4°C.

11. Remove supernatant.

12. Wash the resin 2X with 500 µl 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 supernatant onto the gel.

Sepharose® is a registered trademark of Amersham Pharmacia Biotech, Ltd.
Immunostaining

Immunostaining Protocol

The Anti-V5 and Anti-V5-HRP antibodies can be used in immunohistchemical and immunofluorescent methods to detect fusion proteins in cells or tissues that contain the V5 epitope.

The procedure below describes a general immunostaining protocol for cytopsins, coverslips, or other slide-mounted samples. Other protocols are suitable. For more details (i.e. wash times, temperature), please refer to Antibodies (Harlow and Lane, 1988).

1. Fix cells in 1-3% fresh paraformaldehyde in PBS for 15 minutes.
2. Wash with PBS and permeabilize in 0.2% Triton X-100 in PBS for 5 minutes.
3. Wash with PBS and incubate in PBS containing 1% BSA or bovine serum (blocking buffer) for 30 minutes.
4. Incubate cells with Anti-V5 or the Anti-V5-HRP Antibody diluted 1:200 into blocking buffer for 1 hour.
5. Wash with PBS or blocking buffer.
6. If you are using the Anti-V5 Antibody, add anti-mouse secondary antibody diluted according to the manufacturer’s recommendation into blocking buffer.
7. Wash cells in PBS.
8. If the secondary antibody is conjugated to a fluorescent probe, mount cells in mounting medium containing 0.6% 1,4-diazabicyclo[2.2.2]octane (Sigma, Catalog no. D2522) as an anti-photobleaching agent and view cells under microscope.
9. If anti-mouse secondary antibody is conjugated to an enzyme such as alkaline phosphatase or horseradish peroxidase, follow the manufacturer’s protocol for substrate development and detection.
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5. When you are finished entering information, click the ‘Submit’ button. Your MSDS will be sent within 24 hours.

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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.