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Product Information

Anti-VSV-Glycoprotein-Agarose antibody, Mouse monoclonal
clone P5D4, purified from hybridoma cell culture

Product Number **A1970**

Product Description

Anti-VSV-Glycoprotein-Agarose antibody, Mouse monoclonal is the immunoglobulin fraction of Monoclonal Anti-VSV Glycoprotein (mouse IgG1 isotype) covalently linked to agarose. The monoclonal antibody is purified from hybridoma cell culture of the P5D4 hybridoma using Protein A affinity chromatography, and then immobilized on agarose at 2.0-2.4 mg antibody per ml bed volume.

Recombinant DNA technology enables the insertion of specific sequences to a target gene. These sequences can provide "affinity handles" (tags), which enable the selective identification and purification of the protein of interest.[1-4] The transmembrane glycoprotein of vesicular stomatitis virus (VSV-G) makes a useful tag for recombinant proteins and is an attractive model to study maturation and intracellular transport.

Monoclonal Anti-VSV-Glycoprotein, derived from clone P5D4, recognizes an epitope containing the five carboxy-terminal amino acids of Vesicular stomatitis virus glycoprotein (VSV-G). It recognizes native as well as denatured forms of VSV-G tagged proteins. The product was verified to be active on N-terminal VSV-G tagged fusion proteins expressed in *E. coli* or in mammalian cells. This Anti-VSV-G antibody has been widely used for the study of cell transport processes.[5] In addition, recombinant proteins tagged with the P5D4 epitope have been detected, immunoprecipitated and localized with the antibody.[6,7] Monoclonal Anti-VSV-G-Agarose is useful in purification and identification of expressed VSV-G fusion proteins in bacterial lysates, or in transfected cells.

Reagent

Supplied as a 1:1 suspension (v/v) in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use and extended storage, store at 2-8 °C. Do not freeze**.**

Product Profile

Binding capacity: ≥15 nmole of VSV-G tagged fusion protein per ml of settled resin.

Elution capacity: at least 10 nmole of a VSV-G tagged fusion protein per ml of settled resin, as determined using VSV-G tagged fusion protein .

Note: Optimal binding and elution capacity may vary depending on the protein's molecular weight and the composition of the elution buffers.

Procedures

Procedure for Purification of VSV-G fusion proteins

Pre-equilibrate the column and all buffers and perform all steps at room temperature. To prevent clogging the column, highly viscous samples containing chromosal DNA or RNA should be sonicated or treated with nuclease to reduce the viscosity, and cellular debris and particulate matter must be removed by centrifugation or filtration. In cases where the stability of the protein is temperature sensitive, the following steps may be performed at 2-8 °C.

A. Column Set Up
1. Place the empty chromatography column on a firm support.
2. Attach a drainage tube to the column to control the flow rate. Limit the length of tubing to 25 cm.
3. Remove the top and bottom tabs and rinse the column twice with PBS pH 7.4. Allow the buffer to drain from the column and leave residual PBS in the column to aid in packing the Anti-VSV-Glycoprotein -Agarose

B. Packing the Column

1. Thoroughly suspend the vial of Anti-VSV-Glycoprotein -Agarose to make a uniform suspension of the resin.
2. Immediately transfer the suspension to the column.
3. Allow the agarose bed to drain and rinse the vial with PBS.
4. Add PBS to the column and allow the column to drain again. Do not let the resin bed dry.

C. Washing the Column

Wash the resin with three sequential 5 ml aliquots of glycine-HCl, pH 3.0, followed by three sequential 5 ml aliquots of PBS. Avoid disturbing the agarose bed while loading. Let each aliquot drain completely before adding the next. Do not leave the column in glycine-HCl for more than 20 minutes.

D. Binding of VSV-G Fusion Protein to the Column

1. Load the sample (neutralized to pH 7-8) onto the column under gravity flow, or use a peristaltic pump at a flow rate of 0.5 ml/min.
   Note: Depending upon the protein and flow rate, not all of the protein may bind. Multiple passes over the column or closing the loaded column and incubating it on a rotator for about one hour may improve the binding efficiency.
2. Collect the "flow through" of unbound protein.
3. Wash the column with PBS till $OD_{280} \leq 0.01$.

Select one of the following procedures for elution:

E1. Elution by Glycine-HCl, pH 3.0

Elute the bound VSV-G fusion protein from the column with ten 1 ml aliquots of 0.2 M glycine-HCl, pH 3.0 into vials containing Tris, pH 8-9 for neutralizaton.

Notes: a. Occasionally, low pH may cause the eluted protein to aggregate. In such cases choose an alternative buffer for elution.

b. The column may lose activity after prolonged exposure to low pH.

E2. Elution by VSV-G Peptide

This is a milder elution method. Elute the bound VSV-G tagged fusion protein by five one-column volume aliquots of a solution containing 100 µg/ml VSV-G peptide (Catalog No. V7887) in PBS.

Note: VSV-G peptide has a detectable absorbency at 280 nm and also interferes in other protein determination assays that are based on peptide bonds. Therefore it is recommended to determine the eluted amount by Coomassie staining of SDS-PAGE relative to a known standard.

F. Recycling the Column

It is recommended that the column be regenerated immediately after use by washing with three column volumes of glycine-HCl, pH 3.0. The column should be immediately re-equilibrated in PBS until the effluent is at neutral pH. The number of cycles observed will be dependent on variables such as sample condition.

Note: Do not leave the column in glycine-HCl for longer than 20 minutes.

G. Storing the Column

Wash the column with three column volumes of PBS and store the column at 2-8 °C in PBS containing 15 mM sodium azide.

Procedure for Immunoprecipitation

This procedure is recommended for work with small volumes of resin (20-50 µL).
The work can be performed in 1.5 ml micro-centrifuge tubes or spin columns.

1. Add 40-100 µL of 1:1 suspension of the Anti-VSV-Glycoprotein -Agarose.
2. Pellet the resin by a short spin (12,000g, 30 sec.). Discard the liquid.
3. Wash the resin with PBS or RIPA* buffer, 5 times, 1 ml each. Aspirate traces of final wash.
4. Add clarified bacterial lysate or cell extract to the settled resin. Bring the volume to at least 200 µL with PBS or RIPA if needed.
5. Incubate for 1 hour to overnight on an orbital shaker at 4°C. Shaking must be vigorous enough to suspend the resin.
6. Wash the resin with PBS or RIPA, 4 times 1ml each. After the final wash aspirate the supernatant and leave ~10 µL above the beads.
7. Add 20-50 µL 2X SDS sample buffer. Denature the proteins by heating at 95-100 °C for 3 minutes.
8. Vortex then centrifuge for 5 seconds. Transfer the supernatant to a fresh tube. Load the supernatant into a gel lane and analyze by SDS-PAGE.
9. Detection of the VSV-G tagged fusion protein is best determined by immunoblotting using an antibody to the protein of interest.
   Alternatively, the immunoprecipitated VSV-G tagged proteins can be specifically eluted with VSV-G peptide (Catalog No. V7887). In this case, after the last wash (step 6), incubate the agarose resin with VSV-G peptide for 5 minutes. Spin and collect the supernatant for further steps.
   Recommended concentration is 75 nmol peptide per 100 µL resin.

Note: VSV-G peptide has a detectable absorbency at 280 nm and also interferes in other protein determination assays that are based on peptide bonds. Therefore it is recommended to determine the eluted amount by Coomassie staining of SDS-PAGE relative to a known standard.

* The Anti-VSV-G Agarose is resistant to RIPA buffer (1% sodium deoxycholate, 0.1% SDS, 1% Triton™ X-100, 0.01M Tris-HCl, pH-8, 0.14M NaCl).

References

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