Product Information

Anti-V5 Agarose Affinity Gel

Antibody produced in mouse, purified immunoglobulin, clone V5-10

A7345

Product Description

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes that can provide 'affinity handles' designed to enable the selective identification and purification of the protein of interest.[1-3] The addition of a sequence known as the V5 tag (GKPIPNPLLGLDST) to a given gene creates a stable fusion product that does not appear to interfere with the bioactivity of the protein, or with the biodistribution of the tagged product. Many recombinant proteins have been engineered with the V5-tag. [4-5] Anti-"tag" agarose conjugates may generally facilitate the detection, isolation and purification of these proteins.[6]

Monoclonal Anti-V5 Agarose conjugate recognizes V5-tagged recombinant fusion proteins expressed in transfected mammalian cells. The product may be used for immunoprecipitation assays of V5-tagged fusion proteins from cell lysates. Several dissertations cite use of this product in their research protocols.[7-10]

Anti-V5 Agarose Affinity Gel is made of purified immunoglobulin fraction of monoclonal anti-V5 isolated from ascites fluid of the V5-10 hybridoma coupled to cyanogen bromide-activated beaded agarose. The purified monoclonal antibody is immobilized on agarose, at 1.8 mg to 2.2 mg antibody per mL bed volume. The V5-10 hybridoma was produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a synthetic peptide of 14 amino acids, the 14 amino acids of the V5-tag sequence, as conjugated to maleimide-activated KLH via an *N*-terminal cysteine (C-GKPIPNPLLGLDST).

Reagent

The product is supplied as a 1:1 suspension in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Precautions and Disclaimer

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

1 mL of settled Anti-V5 Agarose Affinity Gel has a binding capacity of at least 2.5 nmoles, as determined using a V5-tagged recombinant fusion protein of 120 kDa expressed in transfected mammalian cells.

Note: Binding and elution capacity may vary, depending on the characteristics of the V5 fusion proteins. For optimal results, it is recommended to try different elution buffers.

Procedures

Binding or Immunoprecipitation assay

General Notes

- Highly viscous samples that contain RNA or chromosomal DNA should be sonicated or treated with nuclease to reduce the viscosity.
- Cellular debris and particulate matter must be removed by centrifugation or filtration prior to binding on the resin.
- Perform all steps at room temperature or at 2-8 °C.
- This procedure is designed for work with small volumes of resin (20-50 µL) and small volumes of extracts expressing the recombinant fusion protein.
- The work can be performed in 1.5 mL microcentrifuge tubes or in spin columns.

1. Add 40 to 100 µL of the 1:1 suspension of the Anti-V5 Agarose conjugate to a microcentrifuge tube or a spin column.
2. Allow the resin to settle by a short spin. Discard the liquid.
3. Wash the resin 5 times with 1 mL PBS (such as Cat. No. D8537).

4. Add clarified lysate or cell extract to the settled resin. Bring the volume to at least 200 µL with PBS or RIPA buffer, if needed.
5. Incubate for 1.5 hours on an orbital shaker. Shaking must be vigorous enough to suspend the resin.
6. Wash the resin 4 times with 1 mL of PBS or lysis buffer (RIPA). **Note**: Anti-V5 Agarose Affinity Gel is resistant to RIPA buffer (1% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, 0.01 M Tris-HCl, pH 8, 0.14 M NaCl).
7. After the final wash, aspirate the supernatant and leave ~10 µL above the beads.
8. Add 20-50 µL 2X SDS sample buffer.
9. Cap the tube or the spin column securely and incubate 5 minutes at 100 °C.
10. Vortex and spin for 5 seconds. Load the supernatant into a gel lane, carefully avoiding the agarose, and analyze by SDS-PAGE.

Analysis of Results: Detection of the V5 tagged fusion protein is performed by immunoblotting, using Rabbit Anti-V5 (Cat. No. V8137).

References

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