Product Information

Anti-V5 Magnetic Beads

Magnetic agarose, suspension

SAE0203

Product Description

In situations where protein-specific antibodies are unavailable, epitope tags enable researchers to study protein topology, characterize and identify new proteins, protein complexes, and associated proteins, even if the proteins of interest are in low abundance or are poorly immunogenic.[4-8]

Monoclonal Anti-V5 (mouse IgG1 isotype) is produced by fusing mouse myeloma cells and splenocytes from a BALB/c mouse that is immunized with a synthetic peptide corresponding to amino acid residues GKPIPNPLLGLDST (95-108) of the P/V proteins of the Paramyxovirus SV5. Increased expression of the cellular oncogene V5 has been described in a variety of human tumors, occurring by several mechanisms, including gene amplification and chromosomal translocation[1-3].

Anti-V5 Magnetic beads are prepared with an affinity purified anti-V5 antibody, produced in mouse (Cat. No. V8012). The antibody is conjugated to cyanogen bromide-activated Magnetic beads at a ~2 mg/mL protein-to-bead ratio.

Anti-V5 recognizes an epitope located on V5-tagged fusion proteins. The antibody reacts specifically with N-terminal and C-terminal V5-tagged fusion proteins and may be used for immunoprecipitation or immunoaffinity purification. Anti-V5 magnetic beads are useful in the purification of expressed V5-tagged fusion proteins from bacterial lysates, mammalian lysates, or transfected cells.

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.

Reagent

The Anti V5-Magnetic beads product is provided as a suspension containing 50% beads, in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide as a preservative.

Storage/Stability

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

Product Profile

Binding capacity: 20-62 nmoles of V5-tagged fusion protein per 1 mL of settled resin

Elution capacity: 5-62 nmol/mL settled resin

Procedure

General Notes

- To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing, or using a rotating platform.
- The following General Procedure is written for use of 20 μL of the anti-V5 magnetic agarose beads, and an estimated bead capacity of 40 μg of V5-tagged protein.
- Our suggestion is to add enough cell lysate where 40 μg of the V5-tagged protein is expected to be present.
- Each sample will differ, depending on the individual researcher's system, and determination or estimation of the degree of V5-tagged protein in the lysate sample.
- A method like SDS-PAGE analysis can be performed on an aliquot of the lysate, with estimated quantitation of lysate protein bands against known amounts of defined protein standards in a separate gel lane.

General Procedure for Purification of V5-tagged Proteins

1. To ensure homogeneity, mix the beads thoroughly before use by repeated inversion, gentle vortexing or using a rotating platform.
2. Pre-washing: Add 20 µL of Anti V5-Magnetic beads into a 1.5 mL microcentrifuge tube.
3. Place the tube into a magnetic stand to collect the beads against the side of the tube.
4. Remove and discard the supernatant. Wash with 200 µL PBS. Repeat the wash 3-5 times.
5. Apply the sample which contains the estimated amount of 40 µg V5-tagged protein. Incubate for 60 minutes at 37 °C using a rotating platform.
6. Collect the flow-through of unbound protein.
7. Wash with PBS until the absorbance at 280 nm is ≤ 0.01.
8. Elution of V5-tagged proteins may be done by incubation with 80 mL of 1× sample buffer for 5 minutes at 95 °C.

Additional Notes

- To obtain the best results in different techniques and preparations, we recommend determining the optimal working concentration by titration test.
- Binding capacity and elution capacity may vary, depending on the characteristics of the V5-tagged fusion proteins. For optimal results, it is recommended to try different elution buffers.

References

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