Product Information

Monoclonal Anti-V5-Peroxidase antibody produced in mouse

Clone V5-10, purified immunoglobulin, lyophilized powder

V2260

Product Description

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes that can provide "affinity handles" or "tags". These tags are intended to enable the selective identification and purification of the protein of interest.[1-3] These protein tag sequences are genetically engineered away from the protein active site, by insertion at the N-terminus or the C-terminus. The addition of a tag sequence such as the V5 sequence (GKPIPNPLLGLDST) does not appear to interfere with the bioactivity or the biodistribution of the recombinant protein.

Monoclonal Anti-V5-Peroxidase is a preparation of the purified immunoglobulin fraction of monoclonal Anti-V5 antibody that has been isolated from ascites fluid of the V5-10 hybridoma, and subsequently conjugated to horseradish peroxidase (HRP). The antibody is derived from the V5-10 hybridoma that is produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse which has been immunized with a KLH-conjugated synthetic 14-mer peptide that corresponds to amino acid residues 95-108 of the P/V proteins of the Paramyxovirus SV5.[4]

Monoclonal Anti-V5-Peroxidase reacts specifically with V5-tagged recombinant fusion proteins expressed in transfected mammalian cells or produced by *in vitro* translation. Several publications[5-6] and dissertations[7-10] cite use of this V2260 product in their protocols.

Reagents

This product is supplied as a lyophilized powder. After reconstitution, the solution contains 1% bovine serum albumin (BSA) and 0.05% MIT (methylisothiazolinone) in 0.01 M sodium phosphate buffered saline (PBS).

Antibody concentration: 5-11 mg/mL

Molar ratio: Ab/Enzyme = 0.6-1.5

Storage/Stability

Store the lyophilized product at 2-8 °C.

Preparation Instructions

Reconstitute the vial with 0.5 mL of distilled water.

- For extended storage after reconstitution, it is suggested to store working aliquots at –20 °C.
- For continuous use after reconstitution, the solution may be stored at 2-8 °C for up to 1 month.
- Working dilutions should be discarded. Avoid repeated freeze-thaw.

Product Profile

Immunoblotting: a working dilution of 1:4,000 to 1:8,000 is determined, using V5-tagged fusion protein that has been expressed in a whole extract of transfected cells or produced by *in vitro* translation, and ECL immunoblotting detection reagent.

Note: To obtain best results in different techniques and preparations, we recommend determining optimal working dilution by titration test.

Procedure

Immunoblotting

All incubation steps should be performed at room temperature.

1. Separate V5-tagged proteins from sample extract using a standard SDS-PAGE protocol. Load an adequate amount of total cell-transfected extract obtained from 10 cm^2 plate per slab.

   Note: The amount of extract to be loaded per slab or lane depends on the level of protein expression, and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.

3. Block the membrane using a solution of 3% non-fat dry milk in PBS (Cat. No. P2194) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS with 0.05% TWEEN® 20 (PBS-T, Cat. No. P3563).
5. Incubate the membrane with Anti-V5 Peroxidase using an optimized concentration in PBS-T and 1% BSA for 60 to 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS-T at room temperature.
7. Treat the membrane with a peroxidase substrate.

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.

References

1. Uhlen, M., and Moks, T., *Methods Enzymol.*, **185**, 129-143 (1990).
2. Kolodziej, P.A., and Young, R.A., *Methods Enzymol.*, **194**, 508-519 (1991).
3. Cravchik, A., and Matus, A., *Gene*, **137(1)**, 139-143 (1993).
4. Thomas, S.M. *et al.*, *Cell*, **54(6)**, 891-902 (1988).
5. Ting, T.C. *et al.*, *Cell Rep.*, **29(6)**, 1499-1510.e6 (2019).
6. Kim, S. *et al.*, *iScience*, **24(3)**, 102146 (2021).
7. Knauf, Mathias M., "Erzeugung und Charakterisierung genetischer Impfstoffe gegen konservierte Proteine von Influenza A" ("Generation and characterization of genetic vaccines against conserved proteins of influenza A"). Freien Universität Berlin, Dr. rer. nat. dissertation, p. 122 (2011).
8. Garcia, Cristiana Bernadelli, "Acúmulo da ribonucleoproteína heterogênea nuclear K em cancer de cabeça e pescoço: estudos mitocondriais" ("Accumulation of heterogeneous nuclear ribonucleoprotein K in head and neck cancer: mitochondrial studies"). Universidade de São Paulo, Ph.D. dissertation, p. 13 (2014).
9. Cruanès, Julie, "A study of the Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) in glucose and insulin homeostasis". McGill University, Ph.D. dissertation, p. 86 (December 2020).
10. Pennemann, Lisa Friederike, "Antiviral properties of species conserved nucleic acid-binding proteins". Technischen Universität München, Dr. rer. nat. dissertation, p. 39 (2021).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

V2260dat Rev 01/22 DS,KAA,PHC,GCY