Product Information

Anti-V5-Cy3™ Antibody, Mouse Monoclonal

Clone V5-10, purified from hybridoma cell culture

V4014

Product Description

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes, which can provide "affinity handles" (tags) designed to enable the selective identification and purification of the protein of interest.[1-3] The addition of a tag 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 to express a short sequence derived from the V5 molecule known as the V5-tag.[4,5] This tag facilitates the detection, isolation and purification of the proteins.6 Monoclonal antibodies reacting specifically with V5 and conjugated to Cy3 may be useful in various immunotechniques, to identify the expression of a V5-tagged fusion protein *in situ* by immunofluorescence.

Monoclonal Anti-V5 (mouse IgG1 isotype) is derived from the V5 hybridoma, which is produced by fusion of mouse myeloma cells and splenocytes from BALB/c mice that have been immunized with a synthetic peptide that corresponds to amino acid residues GKPIPNPLLGLDST (95-108) of the P/V proteins of the Paramyxovirus SV5, conjugated to KLH. The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor, and then conjugated to Cy3. The conjugate is then extensively dialyzed to remove unbound Cy3 fluorophore.

Monoclonal Anti-V5 Cy3 conjugate recognizes the V5 tag sequence on V5-tagged fusion proteins by immunocytochemistry. Several publications7-8 and dissertations[9-12] cite use of this V4014 product in their protocols.

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.

Reagents

The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

F/P Molar Ratio: 3-9

Storage/Stability

For continuous use and extended storage, store at 2-8 °C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

A working concentration between 2.0-4.0 µg/mL detects V5-tagged fusion proteins in transfected mammalian cells by immunofluorescence.

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

Procedure

Direct Immunofluorescent Staining of Cultured Cells

1. Grow transfected cultured cells expressing V5 fusion protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS (such as Cat. No. P3813).
3. Fix the cells with –20 °C methanol (10 minutes), and then with –20 °C acetone (1 minute).
4. Wash coverslips twice in PBS (5 minutes each wash).
5. Incubate coverslips, cell-side-up, with anti-V5 Cy3 conjugate in PBS for 1 hour.

Note: Addition of 1% BSA helps reduce non-specific staining.

6. Wash three times in PBS (5 minutes each wash).

7. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles. Examine using a fluorescence microscope with appropriate filters.

References

1. Olins, P.O., and Lee, S.C., Curr. Opin. Biotechnol., 4(5), 520-525 (1993).
2. Uhlen, M., and Moks, T., Methods Enzymol., 185, 129-143 (1990).
3. Kolodziej, P.A., and Young, R.A., Methods Enzymol., 194, 508-519 (1991).
4. Southern, J.A., et al., J. Gen. Virol., 72(Pt 7), 1551-1557 (1991).
5. Thomas, S.M. et al., Cell, 54(6), 891-902 (1988).
6. Dunn, C. et al., J. Immunol. Meth., 224(1-2), 141-150 (1999).
7. Kalmar, B. et al., Hum. Mol. Genet., 26(17), 3313-3326 (2017).
8. Hufnagel, K. et al., Bio. Protoc., 9(3), e3152 (2019).
9. Li, Xiao, "Drosophila Mage, a component of Smc5/6 DNA response complex, confers resistance to caffeine and genotoxic stress and plays a role in the cell cycle and cell survival". University of Alberta, Ph.D. dissertation, p. 88 (2014).
10. Lueong, Smiths Sengkwakoh, "Trypanosoma brucei: Protein Expression Microarrays and Circulating miRNA during infection". Ruperto-Carola University of Heidelberg, Dr. rer. nat. dissertation, p. 22 (October 2014).
11. Shahryarhesami, Soroosh, "Detection of bacteria and virus-associated Pancreatic Ductal Adenocarcinoma by cell-free protein microarray". Ruprecht-Karls-Universität (Germany), Dr. sc. hum. dissertation, p. 13 (2019).
12. Wu, Yenan, "Identification of transcription factors that specifically bind methylated recognition sites". Ruperto-Carola University of Heidelberg, Dr. rer. nat. dissertation, p. 34 (January 2020).

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