Product Information

Anti-V5 antibody, Mouse monoclonal

Clone V5-10, purified from hybridoma cell culture

V8012

Product Description

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes which can provide 'affinity handles' (tags). These tags are 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 that specifically react with V5 may be useful in various immunotechniques to identify the expression of a V5 fusion protein *in situ,* and by immunoblotting, in bacteria, bacterial lysates or cells and tissues transfected with V5 fusion protein-expressing vectors.

Monoclonal Anti-V5 (mouse IgG1 isotype) 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 synthetic peptide that corresponds to amino acid residues GKPIPNPLLGLDST (95-108) of the P/V proteins of the Paramyxovirus SV5, conjugated to KLH. The isotype is determined using Mouse Monoclonal Antibody Isotyping Reagents (Cat. No. ISO2).

Monoclonal Anti-V5 reacts specifically with V5-tagged recombinant fusion proteins expressed in transfected mammalian cells or produced by *in vitro* translation. The antibody may be used for ELISA, immunoblotting and immunocytochemistry (methanol-acetone fixation).

Reagent

This product is supplied at ~1 mg/mL in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide. The exact concentration is on the specific Certificate of Analysis (CofA) of a given lot number.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Precautions and Disclaimer

Because of the sodium azide content, a Safety Data Sheet for this product has been sent to the attention of the safety officer of your institution. Consult the Safety Data Sheet for information regarding hazardous and safe handling practices.

Product Profile

Immunoblotting

A working concentration of 0.5-1 µg/mL is determined using whole extract of 293T (human embryonal kidney) cells transfected with pcDNA 3.1/V5-His/LacZ plasmid.

Immunocytochemistry

A working concentration of 1-2 µg/mL is determined on methanol-acetone fixed 293T (human embryonal kidney) cells transfected with pcDNA 3.1/V5-His/LacZ plasmid.

Note: To obtain optimal results in different techniques and preparations, we recommend determining the optimal working concentration by titration tests.

Procedure

Procedure for Immunoblotting

Note: All incubations should be performed at room temperature.

1. Separate V5-tagged proteins from sample lysate using a standard SDS-PAGE protocol.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk in PBS for 1 hour.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20.

   Optional: Block with PBS containing 1% BSA for 10 minutes, followed by draining. This may minimize non-specific adsorption of the antibodies.
5. Incubate the membrane with Monoclonal Anti-V5 antibody diluted to 0.5-1.0 µg/mL in PBS containing 0.05% TWEEN® 20, with agitation for 120 minutes.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20.
7. Incubate the membrane with Anti-Mouse IgG (Fab-specific)-Alkaline Phosphatase (such as Cat. Nos. A2179 or A1682) as the secondary antibody, at the recommended concentration in PBS containing 0.05% TWEEN® 20, with agitation for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20.
9. Incubate the membrane in an alkaline phosphatase substrate solution.

Procedure for Indirect Immunofluorescent Staining of Cultured Cells

Note: All incubations should be performed at room temperature (except for Step 3).

1. Grow transfected cultured cells expressing V5-tagged fusion protein on sterile coverslips at 37 °C.
2. Wash the coverslips briefly in PBS.
3. Fix the cells with –20 °C methanol (10 minutes) and then with –20 °C acetone (1 minute).
4. Wash specimens twice in PBS (5 minutes each wash).

   Optional: Block with PBS containing 1% BSA for 10 minutes, followed by draining. This may minimize non-specific adsorption of the antibodies.
5. Incubate specimens cell-side-up for 1 hour with Monoclonal Anti-V5 antibody diluted to 1-2 µg/mL in PBS.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate specimens cell-side-up with Anti-Mouse (Fab specific)-FITC (such as Cat. Nos. F4018 or F8771) as the secondary antibody, at the recommended dilution, in PBS containing 1% BSA, for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of "mounting medium" on the coverslip, and invert it carefully on a glass slide. Avoid air bubbles. Examine using a fluorescence microscope with appropriate filters.

V8012dat Rev 06/21 MG,KAA,PHC,RC,GCY

References

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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).

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V8012dat Rev 06/21 MG,KAA,PHC,RC,GCY