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ProductInformation

ANTI-V5
Developed in Rabbit, IgG Fraction of Antiserum

Product Number **V8137**

Product Description

Anti-V5 is developed in rabbit using a synthetic peptide of 14 amino acids (C-GKPIPNPLLGLDST) conjugated to maleimide activated KLH via an N-terminal cysteine as immunogen. The peptide corresponds to amino acids 95-108 of non-structural protein V and to RNA polymerase α subunit (P protein), of Paramyxovirus SV5.[1] The antibody is an IgG fraction of antiserum.

Anti-V5 reacts specifically with V5 tagged recombinant fusion proteins expressed in transfected mammalian cells or from *in vitro* translation. The antibody may be using in immunoblotting and immunoprecipitation techniques.

Epitope tagging technology enables the insertion of heterologous or synthetic antigenic determinants to proteins. The addition of a tag sequence to a given gene creates a stable fusion product. The tag does not appear to interfere with the bioactivity or the biodistribution of the recombinant protein. The tag can be recognized by a specific antibody and can provide an "affinity handle" designed to enable the selective identification and purification of the expressed recombinant protein of interest.[2-4]

Reagents

Anti-V5 is supplied as a solution of the IgG fraction of antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices

Storage/Stability

Store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. 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.

Immunoblotting Procedure

Note: All incubations should be performed at room temperature.

1. Separate V5 tagged proteins from sample lysate using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (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 at room temperature for 1 hour.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20.
5. Incubate the membrane with Anti-V5 diluted to approx. 2.5 µg/ml in PBS containing 0.05% Tween 20, with agitation for 120 minutes.
   Note: A lower concentration of antibody may be required if chemiluminescent detection is to be used.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05% Tween 20.
7. Incubate the membrane with Anti-Rabbit IgG Alkaline Phosphatase conjugate (Product Code: A9919) 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 with an alkaline phosphatase substrate such as BCIP/NBT (Product Code: B5655) or with chemiluminescent substrate.

Procedure for Indirect Immunofluorescent Staining of Cultured Cells

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-20ºC methanol (10 minutes) and then with –20 ºC acetone (1 minute).
4. Wash specimens twice in PBS, 5 minutes per wash.
5. Incubate specimens at room temperature, cell-side-up with Anti-V5 antibody diluted to approx. 5 µg/ml in PBS for 1 hour.
6. Wash three times in PBS, 5 minutes per wash.
7. Incubate specimens at room temperature, cell-side-up with Anti-Rabbit IgG FITC conjugate (Product Code: F9887) as the secondary antibody at the recommended dilution in PBS containing 1% BSA for 30 minutes.
8. Wash three times in PBS, 5 minutes per 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.

Note:

1. In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.
2. Blocking with PBS containing 1% BSA for 10 minutes at room temperature followed by draining prior to step 5 may minimize non-specific adsorption of the antibodies.

Product Profile

A working concentration of 2.5 µg/ml of the antibody is determined by immunoblotting using V5 tagged fusion protein expressed in whole extracts of transfected cells or *in-vitro* translated.

A concentration of 5 µg/ml specifically stains V5 tagged fusion protein in methanol-acetone fixed transiently transfected 293T (human embryonal kidney) cells by indirect immunofluorescence and 5 µg of antibody will immunoprecipitate V5 tagged fusion protein from extracts of 10^6-10^7 transfected cells.

In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

1. Thomas, S. M., Lamb, R. A. and Paterson, R.G., Cell, **54**, 891-902 (1988).
2. Uhlen, M., and Moks, T., Methods Enzymol., **185**, 129-143 (1990).
3. Kolodziej, P. A., and Young, R .A., Methods Enzmol., **194**, 508 (1991).
4. Cravchick, A., and Matus, A., Gene, **137** 139-143 (1993).

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