3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-Farnesyl
produced in rabbit, whole antiserum

Catalog Number **F6925**

Product Description

Anti-Farnesyl is produced in rabbit using as immunogen N-acetyl-S-farnesyl-L-cysteine conjugated to KLH.

Anti-Farnesyl recognizes farnesyl-cysteine-BSA and it cross-reacts with KLH or geranylgeranyl-cysteine. Applications include the detection and localization of farnesylated or geranylgeranylated forms of proteins by ELISA and immunofluorescence.

Isoprenylation/methylation is an important post-translational lipid modification; the covalent addition of a 15 carbon farnesyl or a 20-carbon geranylgeranyl group to the C-terminus of a protein results in a substantial increase in hydrophobicity. All known G proteins are modified in this way, making the pathway of central interest for an understanding of signal transduction.[1] From nematode[2, 3] to human, many eukaryotic proteins are reported to be isoprenylated.[4] Known prenylated proteins include fungal mating factors, nuclear lamins, Ras and Ras-related GTP-binding proteins (G proteins), and the subunits of trimeric G proteins, protein kinases and at least one viral protein. Prenylation promotes membrane interactions of most of these proteins, which is not surprising given the hydrophobicity of the lipids involved. In addition, prenylation appears to play a major role in several protein-protein interactions. Lin, et al,[5] first employed anti-farnesyl antibody to localize those isoprenylated viral proteins.

Reagent

Supplied as whole antiserum containing 0.1% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at –20 °C. 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.

Procedure

Cells transfected with plasmids encoding an isoprenylated protein, preferentially driven by a CMV promoter, can be stained by the following protocol

1. After appropriate cultivation for gene expression, cells on cover slips are washed two times with cold PBS.
2. Cells are then permeabilized with cold methanol/acetone (1:1) at –20 °C for 5 min.
3. Coverslips are briefly washed with PBS in 6-well plates and incubated in 1% BSA-PBS for 1 hr. at 37 °C (or overnight at 4 °C).
4. Carefully drain the blocking solution from the 6-well plate, leaving the coverslips in the wells. Anti-Farnesyl is diluted 1:30 in 1% BSA-PBS and added to cover the cells on the coverslips (~150 µL/ coverslip).
5. Incubate antibody 1 hr at room temperature without shaking.
6. Cover plates to prevent evaporation.
7. After incubation, the coverslips are washed four times with PBS with gentle shaking (10 min. each).
8. Anti-Rabbit IgG- FITC (typically ~1:500 diluted in 1% BSA-PBS) is added to the coverslips (~150 µl/coverslip).
9. Incubation and subsequent washings are preformed as described above. Protect from light.
10. Coverslips are mounted on glass slides for observation by fluorescent microscope.

Product Profile

Immunofluorescence: a working dilution of 1:40 -1:50 is suggested using cells transfected with plasmids encoding an isoprenylated protein

ELISA: a working dilution of 1:1,000 is determined using farnesyl-cysteine BSA coated at 1 μg/ml

Note: In order to obtain the best results and assay sensitivity in various techniques and preparations, we recommend determining optimal working dilutions by titration.

References

1. Rando, R.R., Chemical biology of protein isoprenylation/methylation. *Biochim. Biophys. Acta*, **1300**, 5-16 (1996).
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3. Gromov, P.S., et al., Identification of isoprenyl modified proteins metabolically labeled with [^{3}H]farnesyl- and [^{3}H]geranylgeranyl-pyrophosphate. *Electrophoresis*, **17 (11)**, 1728-33 (1996).
4. Zhang, F.L., and Casey, P.J., Protein prenylation: molecular mechanisms and functional consequences. *Annual Review of Biochemistry*, **65**, 241-69 (1996).
5. Lin, H.P., et al., Localization of isoprenylated antigen of hepatitis delta virus by anti-farnesyl antibodies. *Journal of General Virology*, **80**, 91-96 (1999).

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