3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

ANTI-l/s-AFADIN
Developed in Rabbit
Affinity Isolated Antibody

Product Number **A 0224**

Product Description

Anti-l/s-Afadin is developed in rabbit using a synthetic peptide corresponding to amino acid residues 1163-1179 of rat afadin, with N-terminal cysteine added. The peptide is conjugated to maleimide-activated bovine serum albumin. The sequence is identical in human. The antibody is affinity-purified using the immunogen peptide immobilized on agarose.

Anti-l/s-Afadin specifically recognizes l-afadin and s-afadin by immunoblotting (approximately 200 and 190 kDa respectively). Additional weak bands may be detected in some extract preparations by immunoblotting. Staining of the l/s-afadin bands is specifically inhibited by the immunizing peptide. The product is also useful for the detection of l/s-afadin by immunocytochemistry and immunohistochemistry. The antibody reacts with l/s-afadin of human, dog, rat, and mouse origin.

Afadin is a cell-cell adherens junction F-actin binding multidomain protein. Two splicing variants of afadin have been described: l-afadin, which is ubiquitously expressed, and the smaller s-afadin, which is abundantly expressed in neural tissue. l-Afadin is a protein containing one PDZ domain in its middle part followed by three proline-rich and one F-actin-binding region at the C-terminal. s-Afadin is a protein which lacks both the third proline-rich and the F-actin binding regions and is homologous to the human AF-6 (ALL-1 fusion partner from chromosome 6) gene product. The AF-6 gene product is fused to the ALL-1 gene in a subset of human acute myeloid leukemia [translocation involving 11q23, t(6;11)(q27;q23)].[1]

l/s-Afadin is localized to cell-cell junctions. It binds several cytoplasmic proteins such as the small GTPase Ras, the tight junction protein ZO-1[2] and the vinculin binding protein ponsin/SH3P12.[3] In addition, l-afadin also binds through its PDZ several integral membrane components such as the Ca^{2+}-independent homophilic immunoglobulin-like nectin, the Junctional Adhesion Molecule (JAM)[4] and a subset of the EphB receptor protein-tyrosine kinases.[5] AF-6/Afadin contains two potential N-terminal Ras-binding protein domains, the first of which was recently shown to interact with Ras and Rap1A GTPases.[6]

Profilin, a key regulator of actin polymerization was recently also shown to interact with AF-6.[6] AF-6/Afadin appears to play a major role in the generation and in the proper organization of adherens junctions and tight junctions. Absence of the AF-6 homologue in mice results in early embryonic lethality most likely due to cell-cell junction disorganization and defects in the embryonic ectoderm polarity.[7]

Reagent

Anti-l/s-Afadin is supplied as a 1.0-1.5 mg/ml solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data 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

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is also 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.

Product Profile

A minimum working dilution of 1:1,000 is determined by immunoblotting (colorimetric) using an extract of rat brain.

A minimum working dilution of 1:250 is determined by indirect immunofluorescent staining of cultured dog (MDCK) cells and of cultured human HepG2 cells.

A minimum working dilution of 1:500 is determined by indirect immunofluorescent staining of frozen sections of mouse liver.

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

References

1. Prasad, R., et al., Cancer Res., **53**, 5624-5628 (1993).
2. Yamamoto, T., et al., J. Cell Biol., **139**, 785-795 (1997).
3. Mandai, K., et al., J. Cell Biol., **139**, 517-528 (1997).
4. Ebnet, K., et al., J. Biol. Chem., **275**, 27979- 27988 (2000).
5. Buchert, M., et al., J. Cell Biol., **144**, 361-371 (1999).
6. Boettner, B., et al., Proc. Natl. Acad. Sci. USA, **97**, 9064-9069 (2000).
7. Ikeda, W., et al., J. Cell Biol., **146**, 1117-1131 (1999).

AH/KAA 01/02

Sigma brand products are sold through Sigma-Aldrich, Inc.
Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.