

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Afadin/AF-6 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human METTL-11A is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human Afadin/AF-6 Asn217-Pro392 Accession # P55196
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

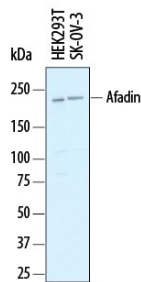
APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. [General Protocols](#) are available in the [Technical Information](#) section on our website.

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	1-15 µg/mL	See Below
Immunohistochemistry	3-15 µg/mL	See Below

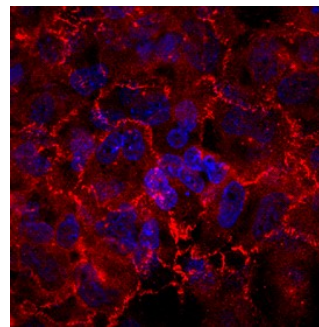
DATA

Western Blot



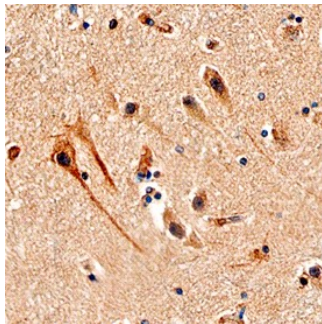
Detection of Human Afadin/AF-6 by Western Blot. Western blot shows lysates of HEK293T human embryonic kidney cell line and SK-OV-3 human ovarian adenocarcinoma cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Afadin/AF-6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7829) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Afadin/AF-6 at approximately 220 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



Afadin/AF-6 in BEAS2B Human Cell Line. Afadin/AF-6 was detected in immersion fixed BEAS2B human lung epithelial cell line using Sheep Anti-Human Afadin/AF-6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7829) at 1.7 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to plasma membrane. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



Afadin/AF-6 in Human Brain. Afadin/AF-6 was detected in immersion fixed paraffin-embedded sections of human brain (hippocampus) using Sheep Anti-Human Afadin/AF-6 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7829) at 3 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to neuronal cell bodies and processes. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Afadin, also MLLT-4 (Myeloid/Lymphoid or mixed-lineage Leukemia Translocated to Ch 4) and AF6, is a 200-210 kDa nectin and F-actin-binding intracellular protein. It is ubiquitously expressed, and serves as an intermediary that links nectin with the actin cytoskeleton in tight and adherens junctions (AJs). Afadin is involved in the nectin-mediated recruitment of catenins to zonule adherens complexes, and in the recruitment of ZO-1 to the apical side of AJs for the purpose of forming tight junctions. Human Afadin is 1824 amino acids (aa) in length. The protein contains two Ras-association (RA) domains (aa 20-348), a phosphoprotein FHA domain (aa 426-492), a Vav2 Ras-activating "dilute" domain (aa 668-908), one PDZ domain that binds protein C-termini (aa 1007-1093), and three proline-rich regions (aa 1346-1708). There are at least fourteen utilized Ser/Thr phosphorylation sites and two Tyr phosphorylation sites. There are at least seven alternative splice variants reported for Afadin. There is a 190 kDa isoform that shows a 12 aa insert after Arg1605 coupled to a four aa substitution for aa 1650-1824. Other isoforms contain scattered deletions of one to three aa coupled to major aa changes. Five isoforms share a deletion of aa 393-407, with four of these containing a 24 aa substitution for aa 1605-1824, a 73 aa substitution for aa 1747-1824, an 11 aa substitution for aa 680-1824, and a deletion of aa 1683-1746, respectively. A final isoform possesses a four aa substitution for aa 1650-1824. Over aa 217-392, human Afadin shares 94% aa sequence identity with mouse Afadin.