

I-Afadin Polyclonal Antibody

Catalog Number PA1-25075

Product data sheet

Details		Species Reactivity	
Size	100 µL	Species reactivity	Dog, Human, Mouse, Rat
Host/Isotope	Rabbit / IgG	<div><div>Tested Applications</div><div>Dilution *</div></div>	
Class	Polyclonal		
Type	Antibody		
Immunogen	Synthetic peptide, conjugated to maleimide-activated KLH, corresponding to residues 1814-1829 of rat I Afadin with an N-terminal added cysteine.		
Conjugate	Unconjugated	Immunohistochemistry (Frozen) (IHC (F))	1:1,000
Form	Liquid	Western Blot (WB)	1:2,000
Concentration	1.2 mg/mL	Immunocytochemistry (ICC/IF)	1:500
Purification	Antigen affinity chromatography	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Storage buffer	0.01M PBS, pH 7.4, with 1% BSA		
Contains	15mM sodium azide		
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.		

Product specific information

Recommended positive controls: MDCK, HepG2, mouse liver, rat brain. Store product as a concentrated solution. Centrifuge briefly prior to opening the vial.

Background/Target Information

AFDN belongs to an adhesion system, probably together with the E-cadherin-catenin system, which plays a role in the organization of homotypic, interneuronal and heterotypic cell-cell adherens junctions (AJs). Nectin- and actin-filament-binding protein that connects nectin to the actin cytoskeleton.

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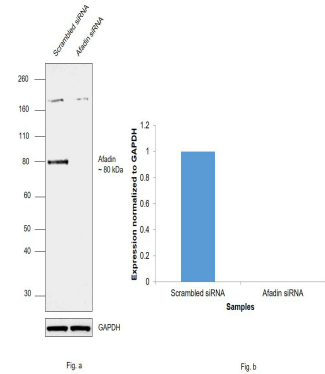
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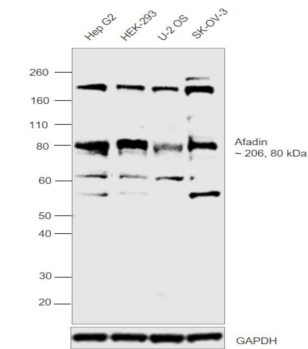
I-Afadin Antibody (PA1-25075)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. Hep G2 cells were transfected with Afadin siRNA and loss of signal was observed in Western Blot using Afadin Polyclonal Antibody (Product # PA1-25075). {KD}



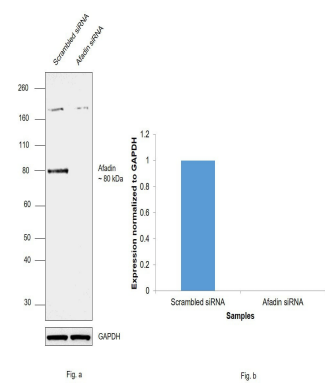
I-Afadin Antibody (PA1-25075) in WB

Western blot was performed using Anti-Afadin Polyclonal Antibody (Product # PA1-25075) and 80, 206 kDa bands corresponding to Afadin was observed across cell lines tested. Whole cell extracts (30 µg lysate) of Hep G2 (Lane 1), HEK-293 (Lane 2), U-2 OS (Lane 3) and SK-OV-3 (Lane 4) were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # LC2001) by XCell SureLock™ Mini-Cell and XCell II™ Blot Module (Product # EI0002). The blot was probed with the primary antibody (1:1000 dilution) and detected by chemiluminescence with Goat Anti-Rabbit IgG Secondary Antibody, HRP conjugate (Product # A27036, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005). An uncharacterized band at ~62 kDa was also observed in the samples tested.



I-Afadin Antibody (PA1-25075) in WB

Knockdown of Afadin was achieved by transfecting Hep G2 with Afadin specific siRNAs (Silencer® select Product # S8829, S8830). Western blot analysis (Fig. a) was performed using whole cell extracts from the Afadin knockdown cells (Lane 2) and non-specific scrambled siRNA transfected cells (Lane 1). The blot was probed with Afadin Polyclonal Antibody (Product # PA1-25075, 1:1000 dilution) and Goat anti-Rabbit IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.25ug/ml, 1:4000 dilution). Densitometric analysis of this Western Blot is shown in histogram (Fig. b). Loss of signal upon siRNA mediated knock down confirms that antibody is specific to Afadin.



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