

PKP1 Monoclonal Antibody (10B2)

Catalog Number32-5700

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human
Host/Isotope	Mouse / IgG1	Published species	Human, Mouse, Not Applicable
Class	Monoclonal	Tested Applications	
Type	Antibody	Immunoprecipitation (IP)	Dilution *2 µg
Clone	10B2	Western Blot (WB)	1-3 µg/mL
Immunogen	N-terminus of Human plakophilin-1.	Immunocytochemistry (ICC/IF)	2 µg/mL
Conjugate	Unconjugated	Published Applications	
Form	Liquid	Immunocytochemistry (ICC/IF)	See 1 publications below
Concentration	0.5 mg/mL	Immunohistochemistry (Frozen) (IHC (F))	See 1 publications below
Purification	Protein A	* 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	PBS, pH 7.4		
Contains	0.1% sodium azide		
Storage Conditions	-20°C		

Background/Target Information

This gene encodes a member of the arm-repeat (armadillo) and plakophilin gene families. Plakophilin proteins contain numerous armadillo repeats, localize to cell desmosomes and nuclei, and participate in linking cadherins to intermediate filaments in the cytoskeleton. This protein may be involved in molecular recruitment and stabilization during desmosome formation. Mutations in this gene have been associated with the ectodermal dysplasia/skin fragility syndrome. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2010].

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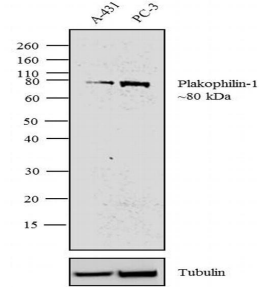
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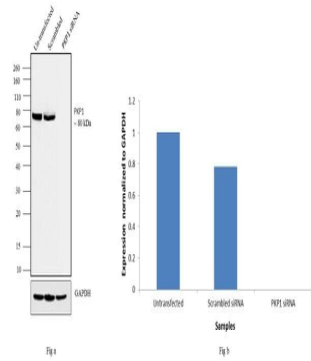
PKP1 Antibody (32-5700) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of A-431 (Lane 1) and PC-3 (Lane 2). The blot was probed with Anti-Plakophilin-1 Mouse Monoclonal Antibody (Product # 32-5700, 2 µg/mL) and detected by chemiluminescence using Goat anti-Mouse IgG (H+L) Superclonal Secondary Antibody, HRP conjugate (Product # A28177, 0.4 µg/mL, 1:2500 dilution). A 80 kDa band corresponding to Plakophilin-1 was observed across the cell lines tested. Known quantity of protein samples were electrophoresed using Novex®NuPAGE®10 % Bis-Tris gel (Product # NP0302BOX), XCell SureLock Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



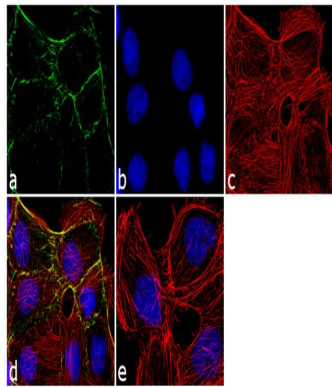
PKP1 Antibody (32-5700)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with DDB1 siRNA and decrease in signal intensity was observed in western blot application (Fig a) using DDB1 Mouse Monoclonal Antibody (Product # 37-6200). Densitometric analysis of this western blot is shown in histogram (Fig b). {KD}



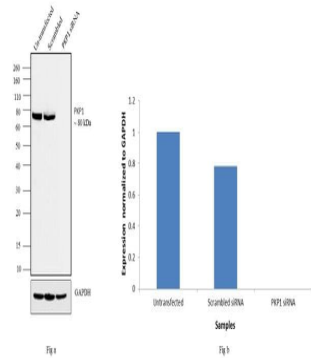
PKP1 Antibody (32-5700) in ICC/IF

Immunofluorescence analysis of Plakophilin (PKP1) was performed using 70% confluent log phase A-431 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with PKP1 (10B2) Mouse Monoclonal Antibody (Product # 32-5700) at 2 µg/mL in 0.1% BSA and incubated for 3 hours at room temperature and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor® 555 Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cell junctional localization. Panel e shows the no primary antibody control. The images were captured at 60X magnification.



PKP1 Antibody (32-5700) in WB

Knockdown of PKP1 was achieved by transfecting HeLa cells with PKP1 specific siRNAs (Silencer® select Product #s10581, s10582). Western blot analysis (Fig a) was performed using whole cell extracts from the PKP1 knock down cells (lane 3), non-specific scrambled siRNA transfected cells (lane 2) and un-transfected cells (lane 1). The blots were probed with Anti-PKP1 Mouse monoclonal Antibody (Product # 37-6200, 2 µg/mL) and Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 µg/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 PKP1.



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PubMed References For PKP1 Monoclonal Antibody (10B2)

1 Immunocytochemistry References

Species / Dilution	Summary
Human / Not Cited	Cell (2003; 113: 249) "Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotrichosis and acquired pemphigus vulgaris."
Mouse / Not Cited	Author(s):Kljuic A,Bazzi H,Sundberg JP,Martinez-Mir A,O'Shaughnessy R,Mahoney MG,Levy M,Montagutelli X,Ahmad W,Aita VM,Gordon D,Uitto J,Whiting D,Ott J,Fischer S,Gilliam TC,Jahoda CA,Morris RJ,Panteleyev AA,Nguyen VT,Christiano AM PubMed Article URL: http://dx.doi.org/10.1016/s0092-8674(03)00273-3

1 Immunohistochemistry (Frozen) References

Species / Dilution	Summary
Human / Not Cited	325700 was used in immunohistochemistry - frozen section to characterize the first female individuals affected with the ectodermal dysplasia/skin fragility syndrome The Journal of investigative dermatology (2004; 122: 647) "Homozygous splice site mutations in PKP1 result in loss of epidermal plakophilin 1 expression and underlie ectodermal dysplasia/skin fragility syndrome in two consanguineous families." Author(s):Sprecher E,Molho-Pessach V,Ingber A,Sagi E,Indelman M,Bergman R PubMed Article URL: http://dx.doi.org/10.1111/j.0022-202X.2004.22335.x

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