abcam

Product datasheet

Anti-Fibronectin antibody [F14] ab45688

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Fibronectin antibody [F14]	
Description	Rabbit monoclonal [F14] to Fibronectin	
Host species	Rabbit	
Tested applications	Suitable for: Flow Cyt (Intra), WB, ICC/IF Unsuitable for: IHC-P	
Species reactivity	Reacts with: Mouse, Rat, Human	
Immunogen	Recombinant full length protein corresponding to Human Fibronectin aa 1-2400. Database link: <u>P02751</u>	
Positive control	WB: Human, mouse and rat serum lysate. IHC-Fr: Rat kidney tissue. ICC/IF: HepG2 cells.	
General notes	 This product is a recombinant monoclonal antibody, which offers several advantages including: High batch-to-batch consistency and reproducibility Improved sensitivity and specificity Long-term security of supply Animal-free production For more information <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>. 	

Properties	
Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.21% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	F14

Abreviews

Applications

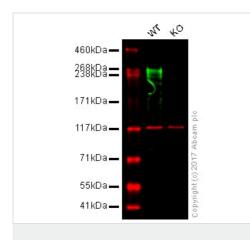
Application

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab45688 in the following tested applications.

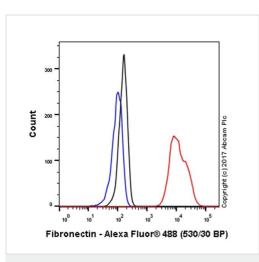
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Notes

Application	Abreviews	Notes	
Flow Cyt (Intra)		1/150.	
WB		1/1000 - 1/10000. Detects a band of approximately 263 kDa (predicted molecular weight: 263 kDa).	
ICC/IF		1/500. For unpurified use at 1/250.	
Application notes	Is unsuitable for IHC-P.		
Target			
Function	Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectins are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Involved in osteoblast compaction through the fibronectin fibrillogenesis cell-mediated matrix assembly process, essential for osteoblast mineralization. Participates in the regulation of type I collagen deposition by osteoblasts. Anastellin binds fibronectin and induces fibril formation. This fibronectin polymer, named superfibronectin, exhibits enhanced adhesive properties. Both anastellin and superfibronectin inhibit tumor growth, angiogenesis and metastasis. Anastellin activates p38 MAPK and inhibits lysophospholipid signaling.		
Tissue specificity	Plasma FN (soluble dimeric form) is secreted by hepatocytes. Cellular FN (dimeric or cross- linked multimeric forms), made by fibroblasts, epithelial and other cell types, is deposited as fibril in the extracellular matrix. UgI-Y1, UgI-Y2 and UgI-Y3 are found in urine.		
Involvement in disease	Glomerulopathy with fibronectin deposits 2		
Sequence similarities	Contains 12 fibronectin type-I domains. Contains 2 fibronectin type-II domains. Contains 16 fibronectin type-III domains.		
	UgI-Y1, UgI-Y2 and UgI-Y3 are present in the urine from 0 to 17 years of age.		
Developmental stage	UgI-Y1, UgI-Y2 and UgI-Y3 ar	e present in the urine from 0 to 17 years of age.	
Developmental stage Post-translational modifications	Sulfated. It is not known whether both of Forms covalent cross-links n glutamine and the epsilon-an heteropolymers (e.g. fibrinog Phosphorylated by FAM20C	or only one of Thr-2064 and Thr-2065 are/is glycosylated. nediated by a transglutaminase, such as F13A or TGM2, between a nino group of a lysine residue, forming homopolymers and en-fibronectin, collagen-fibronectin heteropolymers).	



Western blot - Anti-Fibronectin antibody [F14] (ab45688)

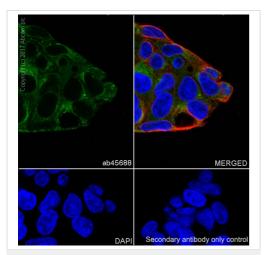


Flow Cytometry (Intracellular) - Anti-Fibronectin antibody [F14] (ab45688) Lane 1: Wild-type HAP1 whole cell lysate (20 µg) Lane 2: Fibronectin knockout HAP1 whole cell lysate (20 µg)

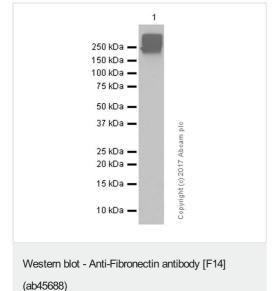
Lanes 1 - 2: Merged signal (red and green). Green - ab45688 observed at 262 kDa. Red - loading control, <u>ab18058</u>, observed at 130 kDa.

ab45688 was shown to react with Fibronectin in wild-type HAP1 cells as signal was lost in Fibronectin knockout cells. Wild-type and Fibronectin knockout samples were subjected to SDS-PAGE. Ab45688 and <u>ab18058</u> (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/500 dilution and 1/2000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling Fibronectin with purified ab45688 at 1/150 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor[®] 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-Fibronectin antibody [F14] (ab45688) Immunocytochemistry/ Immunofluorescence analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling Fibronectin with Purified ab45688 at 1:500 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1:200. **ab150077** Goat anti rabbit IgG(Alexa Fluor[®] 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



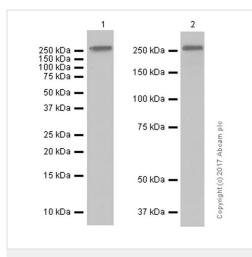
Anti-Fibronectin antibody [F14] (ab45688) at 1/10000 dilution (purified) + Human serum lysates at 15 μg

Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 263 kDa

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-Fibronectin antibody [F14] (ab45688) **All lanes :** Anti-Fibronectin antibody [F14] (ab45688) at 1/10000 dilution (purified)

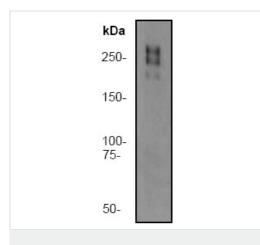
Lane 1 : Mouse serum lysates Lane 2 : Rat serum lysates

Lysates/proteins at 15 µg per lane.

Secondary

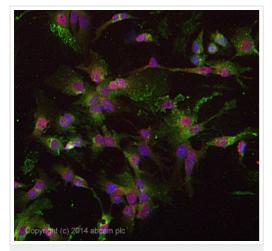
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 263 kDa Observed band size: 263 kDa Blocking and diluting buffer: 5% NFDM/TBST



Western blot - Anti-Fibronectin antibody [F14] (ab45688) Anti-Fibronectin antibody [F14] (ab45688) at 1/5000 dilution (unpurified) + human serum

Predicted band size: 263 kDa Observed band size: 263 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Fibronectin antibody [F14] (ab45688) ICC/IF image of unpurified ab45688 stained HepG2 (Human liver hepatocellular carcinoma cell line) cells. The cells were fixed in 4% formaldehyde (10 minutes) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab45688 at 1/250 dilution overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor[®] 488 goat antirabbit (**ab150081**) IgG (H+L) preadsorbed, used at a 1/1000 dilution for 1 hour. Alexa Fluor[®] 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (pseudocolored blue) at a concentration of 1.43 µM for 1 hour at room temperature.

Why choose α recombinant antibody? Research with Long-term and confidence scalable supply Consistent and Recombinant reproducible results technology Success from the Ethical standards first experiment compliant Confirmed Animal-free specificity production

Anti-Fibronectin antibody [F14] (ab45688)

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