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Datasheet for 600-401-117-0.1

Fibronectin Antibody

Overview

Description:	Anti-Fibronectin (Human) (RABBIT) Antibody - 600-401-117-0.1
Item No.:	600-401-117-0.1
Size:	100 µg
Applications:	IF, IHC, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Human fibronectin has a molecular weight of 450,000 daltons when purified in an intact form from plasma. Fibronectin is a glycoprotein synthesized in the liver for the circulating blood plasma form, and is synthesized by many mesenchymal cells, for the extracellular matrix form. It is composed of two similar, but not identical protein chains, which are linked by two disulfide linkages at the C-terminal end of the chains. The chains are composed of domains which have specific secondary structures linked together by regions which are especially susceptible to proteolytic action. For this reason, detection by immunoblot (western) may show considerable variability in the observed apparent molecular weights, which is predicated on the source of the fibronectin, and to what degree proteolysis has occurred. Bands approximately 225 kDa should be observed after SDS-PAGE when reducing and denaturing conditions are used.
Synonyms:	rabbit anti-Fibronectin antibody, FN1, FN, Cold-insoluble globulin, CIG, Anastellin, Ugl-Y1, Ugl-Y2, Ugl-Y3
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	FN1
Reactivity:	Human
Immunogen Type:	Native Protein

Immunogen:	Fibronectin was purified from Human plasma by binding to a denatured gelatin column followed by elution with high concentrations of arginine. The eluted material was further purified by gel filtration. Immunization occurred after single-band purity was assessed by SDS-PAGE.
Purity/Specificity:	This product has been prepared by immunoaffinity chromatography using immobilized antigens followed by extensive cross-adsorption against human serum proteins and collagen and non-collagen extracellular matrix proteins to remove any unwanted specificities. Typically less than 1% cross reactivity against other extracellular matrix proteins was detected by ELISA against purified standards. This antibody reacts with human Fibronectin and has negligible cross-reactivity with Type I, II, III, IV, V or VI Collagens or Laminin. Non-specific cross reaction of anti-Fibronectin antibodies with other human serum proteins or non-Fibronectin extracellular matrix proteins is negligible.
Relevant Links:	• 600-401-117 SDS • NCBI - AAA53376.1 • NCBI - P02751.4 • UniProtKB - P02751 • GeneID - 2335

Application Details

Suggested Applications:	IF, IHC, WB (Based on references)
Application Note:	Anti-Fibronectin antibodies have been used for indirect trapping ELISA for quantitation of antigen in serum using a standard curve, for immunoprecipitation and for western blotting for highly sensitive qualitative analysis. Rockland's anti-Fibronectin detects intact fibronectin (Invitrogen, Cat. No. 33016-015) by western blot after digestion by Matrix Metalloproteinase-3 (MMP-3) overnight at 37° C. Separation was performed using a 4-12% Tris-Glycine gel. Under these conditions a sizeable, dark band at ~220 kDa representing the undigested fibronectin, as well as many, smaller bands representing the variably sized fragments resulting from fibronectin digestion by MMP-3 were noted. For immunohistochemistry paraffin embedded tissue preparation is recommended.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:5,000 - 1:10,000
IHC:	1:50 - 1:200
IP:	1:100
WB:	1:5,000 - 1:10,000

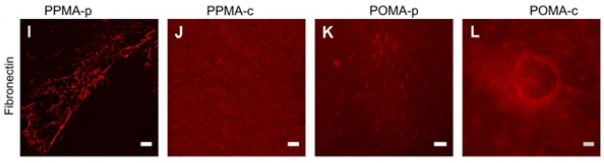
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

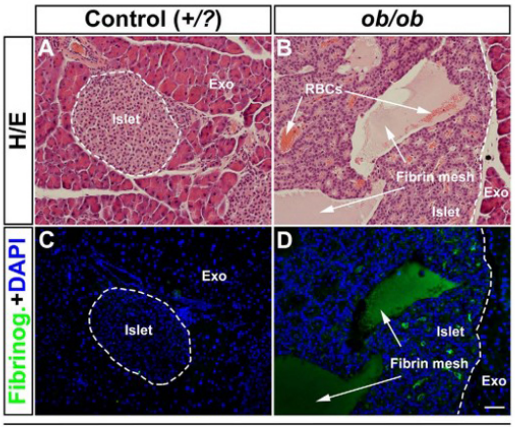
Shipping & Handling

Shipping Condition:	Wet Ice
Storage Condition:	Store vial at 4° C prior to opening. This product is stable at 4° C as an undiluted liquid. Dilute only prior to immediate use. For extended storage mix with an equal volume of glycerol, aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

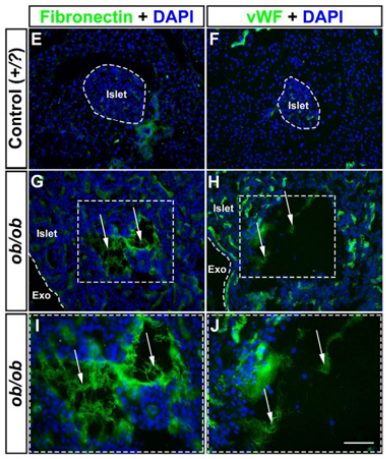
Images



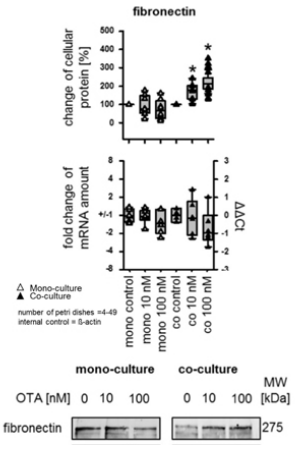
Immunofluorescence Microscopy
Immunofluorescence of Anti-Fibronectin Antibody. Representative images of isolated EC cultured on various MA-coated surfaces after 24 h exposure to 0.5 dyn/cm2 immunofluorescence-labelled for Fibronectin. A strong rearrangement of the initial Fibronectin layer into coarse fibrils (under venous shear stress) (Fig. 3I) with severe displacements of Fibronectin occurred on PPMA-p. Only slight Fibronectin reorganization into fine fibrils (PPMA-c Fig. 3J) or no Fibronectin reorganization at all (POMA-p and POMA-c Fig. 3K and L) were observed as expected from the higher Fibronectin anchorage strength to these latter substrates in comparison to PPMA-p. These findings are in line with earlier results at static cell culture conditions of isolated EC [4e6] showing the dependence of adhesion and stress fibre patterns on the matrix anchorage to the polymer surface, which were now attenuated by the application of shear stress.
Scale bar: 10 mm. Fig. 3. PMID: 22154622.



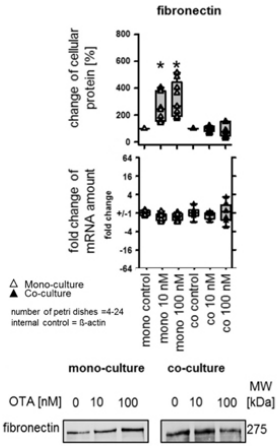
Immunofluorescence Microscopy
Immunohistochemistry of Anti-Fibronectin Antibody.
Immunohistochemical assessment of proteins involved in blood coagulation in ob/ob pancreas. (A,B) Hematoxylin/Eosin staining of an islet from a lean control (A) and a ob/ob (B) pancreas at 52 weeks. Note the accumulation of RBCs (white arrows in (B). (C,D) Consecutive sections to (A,B) stained for Fibrinogen (green) and DAPI (Blue) indicating the presence of a fibrin mesh within the areas of the lesions (white arrows in (D) compare with (B)). Scale bar in (D) is 50µm. Figure 6. PMID: 27713548.



Immunofluorescence Microscopy
Immunohistochemistry of Anti-Fibronectin Antibody.
Immunohistochemical assessment of proteins involved in blood coagulation in ob/ob pancreas. (E–J) Photomicrographs of representative pancreatic cryosections from lean control (E,F) and ob/ob (G,H) pancreas at 52 weeks of age labeled for Fibronectin (Green E,G) and von Willebrand Factor (Green, F,H) together with DAPI (blue). Areas enclosed by a broken line in (G,H) corresponds to (I,J) respectively. The areas in the lesions positive for Fibronectin and von Willebrand factor are not associated with any nucleated cells. Abbreviations; vWF, von Willebrand Factor; Exo, Exocrine tissue. Scale bar in (J) is 92µm in (E–H) and 50µm in (I,J). Figure 6. PMID: 27713548.

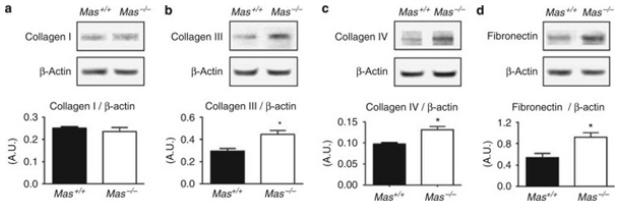


Western Blot
Western Blot of Anti-Fibronectin Antibody.
Impact of OTA on the cellular protein and mRNA amount in renal epithelial cells [NRK-52E]. OTA effect on cellular fibronectin protein amount and mRNA abundance in NRK-52E under mono- and co-culture conditions. Representative Western blots of proteins isolated from cells exposed to OTA. * indicates significant difference compared with the control group.
In the presence of fibroblasts, exposure to 10 nM OTA led to an increase of fibronectin protein amount. Incubation with 100 nM OTA led to an increase of fibronectin protein amount. (Fig. 3 only fibronectin displayed). Fig. 3. PMID: 31415839.



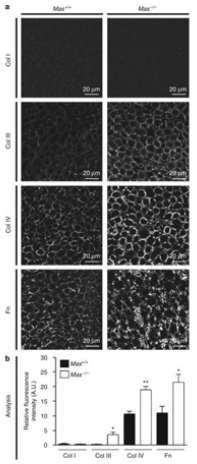
Western Blot

Western Blot of Anti-Fibronectin Antibody.
Impact of OTA on cellular protein and mRNA amount in fibroblasts [NRK-49F]. OTA effect on fibronectin protein amount and mRNA abundance in NRK-49F under mono and co-culture conditions. Representative Western blots of proteins isolated from cells exposed to OTA. * indicates significant difference compared with the control group. Exposure of fibroblasts in monoculture to 10 or 100 nM OTA caused an increase of fibronectin protein amount. Fig. 4. PMID: 31415839.



Western Blot

Immunoblotting of extracellular matrix (ECM) proteins in kidneys of Mas+/+ and Mas−/− animals. (a) Immunoblotting shows no difference of Collagen I (p/n 600-401-103) expression in Mas+/+ and Mas−/− mice kidneys. Significant increases in (b) Collagen III (p/n 600-401-105), (c) Collagen IV (p/n 600-401-106), and (d) fibronectin (p/n 600-401-117) expression were detected by comparing immunoblots of Mas−/− mouse kidneys with those of Mas+/+ controls. Each band represents one mouse kidney from either Mas+/+ or Mas−/− mice. Data are shown as the mean±s.e.m. *P<0.05. A.U. indicates arbitrary unit. Fig 5. PMID: 19262461



Immunofluorescence Microscopy

Immunofluorescence of extracellular matrix (ECM) proteins in the medulla of kidneys from Mas+/+ (left column) and Mas−/− (right column) mice. (a) Fluorescence (Cy3-labeled anti-rabbit IgG) reveals the immunolabeling of ECM proteins. Expression of type III collagen (Col III) (p/n 600-401-105), type IV collagen (Col IV) (p/n 600-401-106), and fibronectin (Fn) (p/n 600-401-117) were increased in the medulla of Mas−/− compared with that of Mas+/+ mice, whereas the expression of type I collagen (Col I) (p/n 600-401-103) was unaltered. (b) Quantification of ECM proteins in the medulla of Mas+/+ and Mas−/− mice. Data are shown as mean±s.e.m. *P<0.05; **P<0.01. A.U. indicates arbitrary unit. Fig 3. PMID: 19262461

References

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