# **Anti-Fibronectin Antibody [PSH14-21]**

#### **HA723610**



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse

Applications: WB, IHC-P, IF-Cell, FC

Molecular Wt: Predicted band size: 272 kDa

Clone number: PSH14-21

Description: Fibronectin is an extracellular matrix glycoprotein present on most cell surfaces, in

extracellular fluids and in plasma. A high molecular weight heterodimeric protein, it was originally discovered as a protein missing from the surfaces of virus-transformed cells, and it has been shown to be involved in various functions including cell adhesion, cell motility and wound healing. Alternative splicing and glycosylation give rise to several different forms of Fibronectin, some of which exhibit restricted tissue distribution or association with malignancies. It has been shown that Myofibroblast phenotype formation correlates with the occurrence of glycosylated Fibronectin and Fibronectin splice variants in Dupuytren's

disease.

Positive control: HepG2 cell lysate, NIH/3T3 cell lysate, human kidney tissue, human stomach tissue, mouse

kidney tissue, mouse stomach tissue, HepG2, NIH/3T3.

**Subcellular location:** Secreted, extracellular space, extracellular matrix.

Database links: SwissProt: P02751 Human | P11276 Mouse

**Recommended Dilutions:** 

**WB** 1:5,000

IHC-P 1:200-1:1,000 IF-Cell 1:5,000-1:50,000

**FC** 1:5,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

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**Images** 

kDa kee kinnis 5 kDa kee kinnis 5 250-150-100-75-55-45-35-25**Fig1:** Western blot analysis of Fibronectin on different lysates with Rabbit anti-Fibronectin antibody (HA723610) at 1/5,000 dilution.

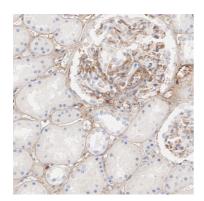
Lane 1: HepG2 cell lysate Lane 2: NIH/3T3 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 272 kDa Observed band size: 272 kDa

Exposure time: 30 seconds; ECL: K1801;

4-20% SDS-PAGE gel.



**Fig2:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Fibronectin antibody (HA723610) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA723610) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

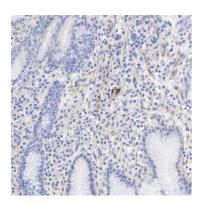
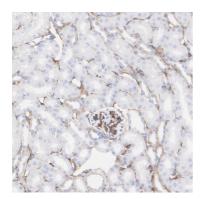


Fig3: Immunohistochemical analysis of paraffin-embedded human stomach tissue with Rabbit anti-Fibronectin antibody (HA723610) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH $_2$ O and PBS, and then probed with the primary antibody (HA723610) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

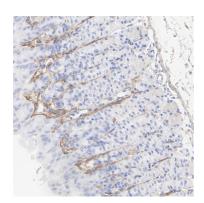
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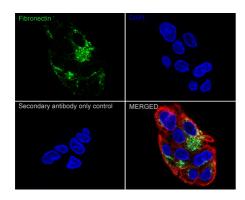
**Fig4:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Fibronectin antibody (HA723610) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA723610) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded mouse stomach tissue with Rabbit anti-Fibronectin antibody (HA723610) at 1/1,000 dilution.

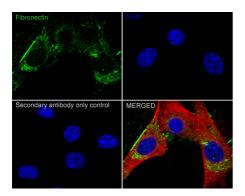
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH $_2$ O and PBS, and then probed with the primary antibody (HA723610) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** Immunocytochemistry analysis of HepG2 cells labeling Fibronectin with Rabbit anti-Fibronectin antibody (HA723610) at 1/50,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Fibronectin antibody (HA723610) at 1/50,000 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor  $\pm$  594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



**Fig7:** Immunocytochemistry analysis of NIH/3T3 cells labeling Fibronectin with Rabbit anti-Fibronectin antibody (HA723610) at 1/5,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Fibronectin antibody (HA723610) at 1/5,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor  $^{\dagger}$  594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

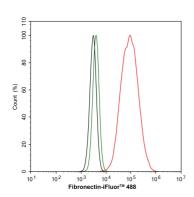


Fig8: Flow cytometric analysis of NIH/3T3 cells labeling Fibronectin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723610, 1/5,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- 1. Patten J et al. Fibronectin in development and wound healing. Adv Drug Deliv Rev. 2021 Mar
- 2. Dalton CJ et al. Fibronectin: Molecular Structure, Fibrillar Structure and Mechanochemical Signaling. Cells. 2021 Sep

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