Anti-Fibronectin Antibody [JF0582]

ET1702-25

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 272 kDa
Clone number:	JF0582

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.

Immunogen: Recombinant full length protein of Human Fibronectin aa 1-2477 / 2477.

Positive control: HepG2 cell lysate, Caco-2 cell lysate, SK-MEL-28 cell lysate, human kidney tissue lysate, MG-63 cell lysates, HepG2, Hela, NIH/3T3, mouse liver tissue.

Subcellular location: Extracellular matrix.

Database links: SwissProt: P02751 Human | P11276 Mouse

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

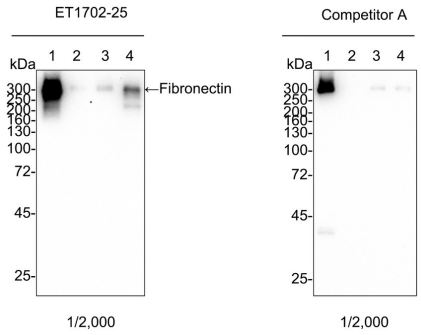

Fig1: Western blot analysis of Fibronectin on different lysates with Rabbit anti-Fibronectin antibody (ET1702-25) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: Caco-2 cell lysate
Lane 3: SK-MEL-28 cell lysate
Lane 4: Human kidney tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 3 minutes 20 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-25) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

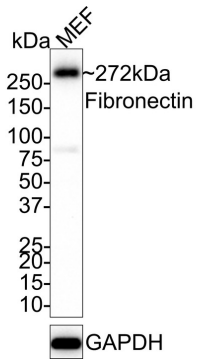

Fig2: Western blot analysis of Fibronectin on MEF cell lysates with Rabbit anti-Fibronectin antibody (ET1702-25) at 1/1,000 dilution.

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 25 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-25) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

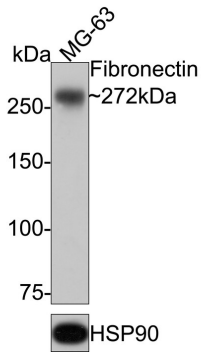

Fig3: Western blot analysis of Fibronectin on MG-63 cell lysates with Rabbit anti-Fibronectin antibody (ET1702-25) at 1/1,000 dilution.

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 2 minutes;

6% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-25) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

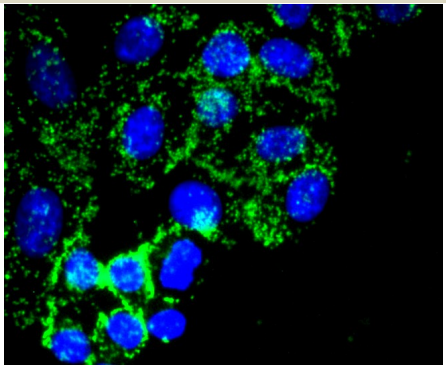

Fig4: ICC staining of Fibronectin in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-25, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

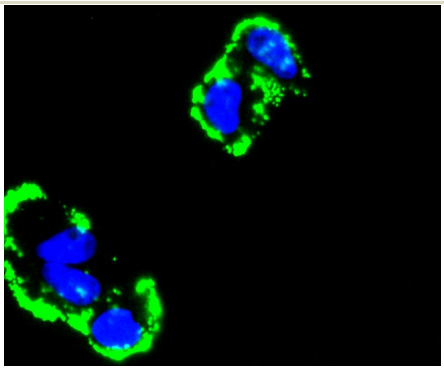

Fig5: ICC staining of Fibronectin in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-25, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

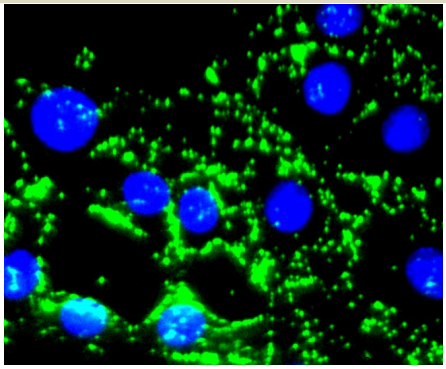

Fig6: ICC staining of Fibronectin in NIH/3T3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-25, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

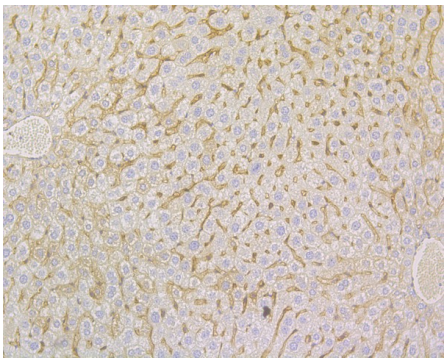

Fig7: Immunohistochemical analysis of paraffin-embedded mouse liver tissue using anti-Fibronectin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-25, 1/50) for 30 minutes 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.

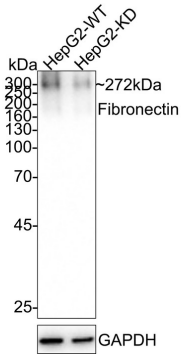

Fig8: Western blot analysis of Fibronectin on different lysates with Rabbit anti-Fibronectin antibody (ET1702-25) at 1/2,000 dilution.

Lane 1: HepG2-si NT cell lysate
Lane 2: HepG2-si Fibronectin cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 20 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-25) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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

Background References

1. Li C et al. RhoA determines lineage fate of mesenchymal stem cells by modulating CTGF-VEGF complex in extracellular matrix. Nat Commun 7:11455 (2016).
2. Beach JA et al. Sphingosine kinase 1 is required for TGF- mediated fibroblastto- myofibroblast differentiation in ovarian cancer. Oncotarget 7:4167-82 (2016).