Anti-Fascin Antibody [JM12-53]

ET1705-18

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	JM12-53

Description: Fascin is an actin bundling protein. Fascin binds beta-catenin,and colocalizes with it at the leading edges and borders of epithelial and endothelial cells. Fascin localizes to actin-rich protrusions at the cell surface called filopodia. T regulatory cell adhesion to antigen presenting dendritic cell causes sequestration of Fascin-1, an actin-bundling protein essential for immunological synapse formation, and skews Fascin-1–dependent actin polarization in antigen presenting dendritic cells toward the T reg cell adhesion zone. Although it is reversible upon T regulatory cell disengagement, this sequestration of essential cytoskeletal components causes a lethargic state of dendritic cells, leading to reduced T cell priming. This suggests Treg-mediated suppression of antigen presenting cells is a multi-step process. In addition to CTLA-4 CD80/CD86 interaction fascin dependent polarization of cytoskeleton towards dendritic cell Treg immune synapse play a pivotal role. In normal tissue, inflammation and the immune response would be limited by secretion of TGF-β. TGF-β on the one hand induces fascin expression, but on the other hand, restricts activity of transcription factor NF-κB. This results to limited fascin expression and allows tissue to rebuild epithelial barriers. In cancer, instead, TGF-β does not restrict NF-κB activity, and both can increase fascin expression, disrupting tissue structure and function.

Immunogen: Recombinant protein within Human Fascin aa 221-389 / 493.

Positive control: HeLa cell lysate, Mouse brain tissue lysate, Mouse kidney tissue lysate, Mouse heart tissue lysate, Rat brain tissue lysate, SH-SY5Y, human kidney tissue, rat brain tissue, human tonsil tissue.

Subcellular location: Cytoskeleton, stress fiber, cytosol, cell cortex, filopodium, invadopodium, microvillus, cell junction.

Database links: SwissProt: Q16658 Human | Q61553 Mouse | P85845 Rat

Recommended Dilutions:

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

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

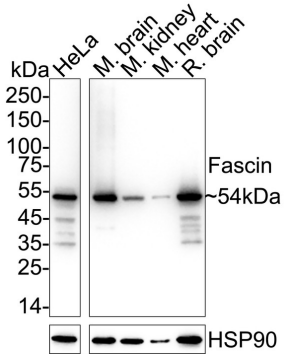

Fig1: Western blot analysis of Fascin on different lysates with Rabbit anti-Fascin antibody (ET1705-18) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: Mouse brain tissue lysate
Lane 3: Mouse kidney tissue lysate
Lane 4: Mouse heart tissue lysate
Lane 5: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: 4 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 (ET1705-18) 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.

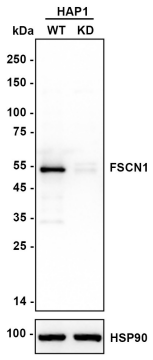

Fig2: Western blot analysis of Fascin on different lysates with Rabbit anti-Fascin antibody (ET1705-18) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Fascin KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: 4 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 (ET1705-18) at 1/1,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

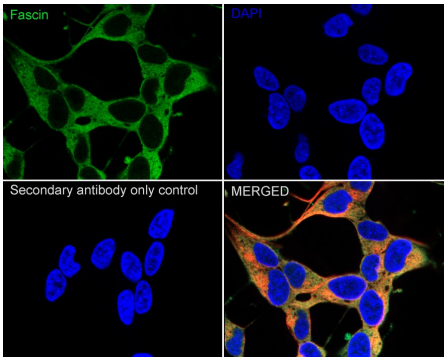

Fig3: Immunocytochemistry analysis of SH-SY5Y cells labeling Fascin with Rabbit anti-Fascin antibody (ET1705-18) at 1/2,000 dilution.

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-Fascin antibody (ET1705-18) at 1/2,000 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

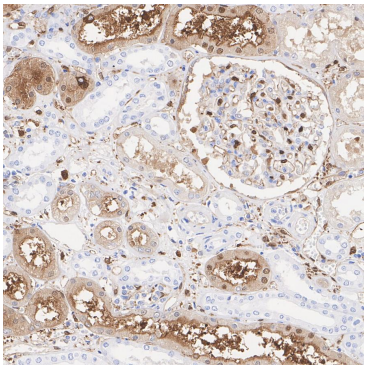

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Fascin antibody (ET1705-18) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1705-18) 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.

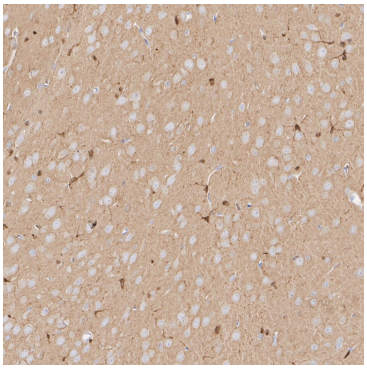

Fig5: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Fascin antibody (ET1705-18) at 1/1,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1705-18) 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.

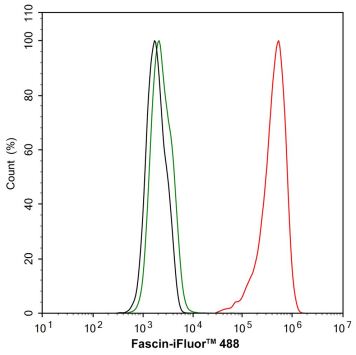

Fig6: Flow cytometric analysis of SH-SY5Y cells labeling Fascin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-18, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Gao W. et. al. Promoter Methylation-Regulated miR-145-5p Inhibits Laryngeal Squamous Cell Carcinoma Progression by Targeting FSCN1. Mol Ther. 2019 Feb
2. Ma L. et. al. miR-145 Contributes to the Progression of Cervical Carcinoma by Directly Regulating FSCN1. Cell Transplant. 2019 Sep-Oct