

Anti-Villin1 Antibody [JU34-75]

ET7106-62



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, mIHC
Molecular Wt:	Predicted band size: 93 kDa
Clone number:	JU34-75

Description: Caldesmon, Filamin 1, Nebulin and Villin are differentially expressed and regulated Actin binding proteins. Both muscular (CDh) and non-muscular (CDI) forms of Caldesmon have been identified and each has been shown to bind to Actin as well as to calmodulin and myosin. CDh is expressed predominantly on thin filaments in smooth muscle, whereas CDI is widely expressed in non-muscle tissues and cells. Filamin 1, which is ubiquitously expressed and exists as a homodimer, functions to crosslink Actin to filaments. Nebulin is a large filamentous protein specific to muscle tissue that may function as a ruler for filament length. Several isoforms of Nebulin are produced by alternative exon usage. Villin is Ca²⁺-regulated and is the major structural component of the brush border of absorptive cells.

Immunogen: Synthetic peptide within Human Villin1 aa 176-225 / 827.

Positive control: Caco-2 cell lysate, COLO205 cell lysate, AGS cell lysate, Mouse kidney tissue lysate, Mouse colon tissue lysate, Rat kidney tissue lysate, Rat colon tissue lysate, Hela, HepG2, LOVO, human colon cancer tissue, human kidney tissue, mouse colon tissue.

Subcellular location: Cytoskeleton.

Database links: SwissProt: P09327 Human | Q62468 Mouse
Entrez Gene: 316521 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IF-Cell	1:500-1:2,000
IHC-P	1:50-1:200
FC	1:50-1:100
mIHC	1:5,000

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

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

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

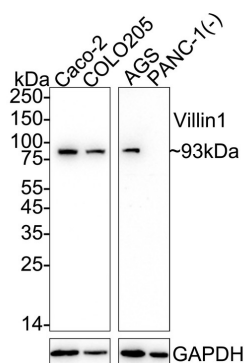


Fig1: Western blot analysis of Villin1 on different lysates with Rabbit anti-Villin1 antibody (ET7106-62) at 1/5,000 dilution.

Lane 1: Caco-2 cell lysate (20 µg/Lane)

Lane 2: COLO205 cell lysate (20 µg/Lane)

Lane 3: AGS cell lysate (20 µg/Lane)

Lane 4: PANC-1 cell lysate (negative) (20 µg/Lane)

Predicted band size: 93 kDa

Observed band size: 93 kDa

Exposure time: Lane 1-2: 16 seconds; Lane 3-4: 3 minutes; 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 (ET7106-62) at 1/5,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

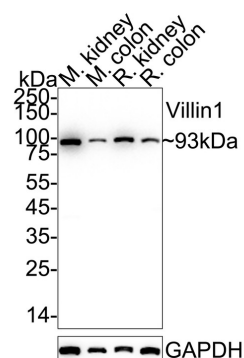
Fig2: Western blot analysis of Villin1 on different lysates with Rabbit anti-Villin1 antibody (ET7106-62) at 1/2,000 dilution.

Lane 1: Mouse kidney tissue lysate

Lane 2: Mouse colon tissue lysate

Lane 3: Rat kidney tissue lysate

Lane 4: Rat colon tissue lysate



Lysates/proteins at 20 µg/Lane.

Predicted band size: 93 kDa

Observed band size: 93 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 (ET7106-62) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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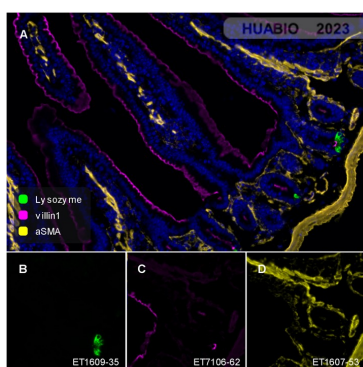


Fig3: Fluorescence multiplex immunohistochemical analysis of mouse small intestine (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Lysozyme (ET1609-35, Green), anti-villin1 (ET7106-62, Magenta) and anti-aSMA (ET1607-53, Yellow) on mouse small intestine. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1609-35 (1/2,000 dilution), ET7106-62 (1/5,000 dilution) and ET1607-53 (1/10,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95°C. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

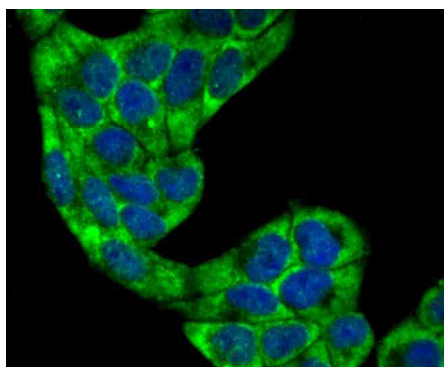


Fig4: ICC staining of Villin1 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-62, 1/500) for 1 hour at 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).

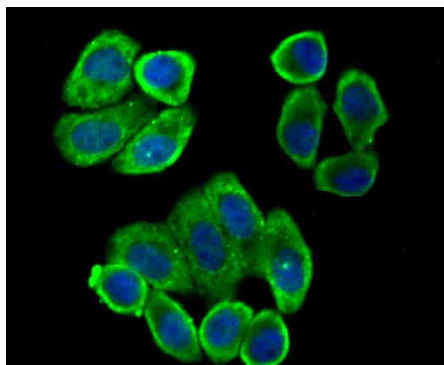


Fig5: ICC staining of Villin1 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-62, 1/500) for 1 hour 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).

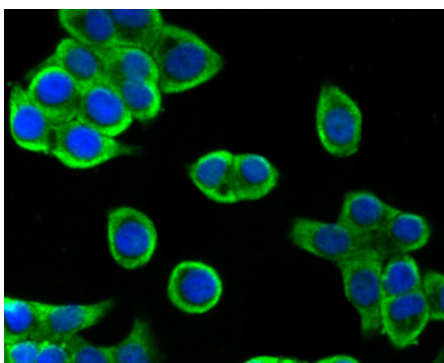


Fig6: ICC staining of Villin1 in LOVO cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-62, 1/500) for 1 hour at 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).

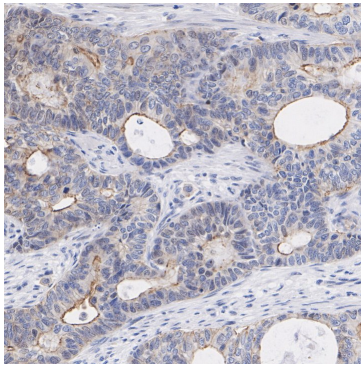


Fig7: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Villin1 antibody (ET7106-62) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET7106-62) 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.

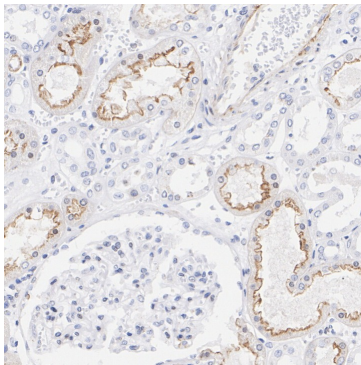


Fig8: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Villin1 antibody (ET7106-62) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ET7106-62) 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.

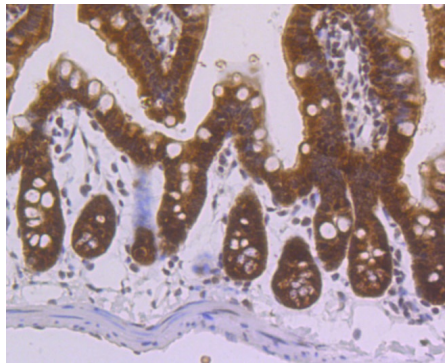


Fig9: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-Villin1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET7106-62, 1/200) 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.

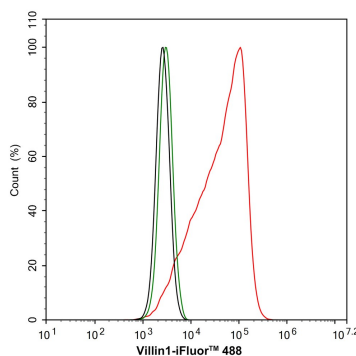


Fig10: Flow cytometric analysis of HeLa cells labeling Villin1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET7106-62, 1/1,000) (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. Northrop J et al. Different calcium dependence of the capping and cutting activities of villin. J Biol Chem 261:9274-9281 (1986).
2. Zhai L et al. Tyrosine phosphorylation of villin regulates the organization of the actin cytoskeleton. J Biol Chem 276:36163-36167 (2001).

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