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:	92/46 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 (CDl) 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 CDl 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: Mouse colon tissue lysate, human small intestine tissue lysate, human colon tissue lysate, rat kidney tissue lysate, Hela, HepG2, LOVO, human colon carcinoma tissue, human kidney tissue, mouse colon tissue, mouse small intestine.

Subcellular location: Cytoskeleton.

Database links: SwissProt: P09327 Human | Q62468 Mouse

Recommended Dilutions:

WB	1:500-1:2,000
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: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

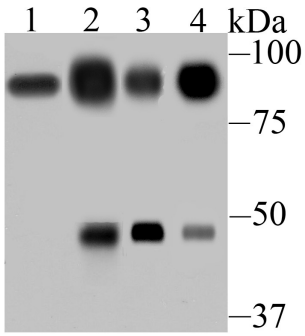

Fig1: Western blot analysis of villin1 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET7106-62, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Mouse colon tissue lysate
Lane 2: Human small intestine tissue lysate
Lane 3: Human colon tissue lysate
Lane 4: Rat kidney tissue lysate

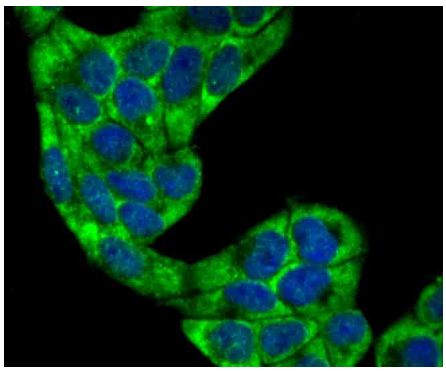

Fig2: ICC staining of villin1 in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-62, 1/500) for 1 hour at 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).

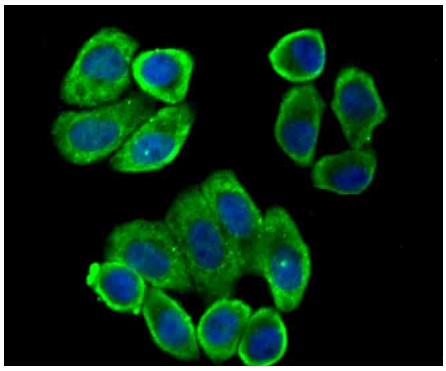

Fig3: ICC staining of villin1 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-62, 1/500) for 1 hour 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).

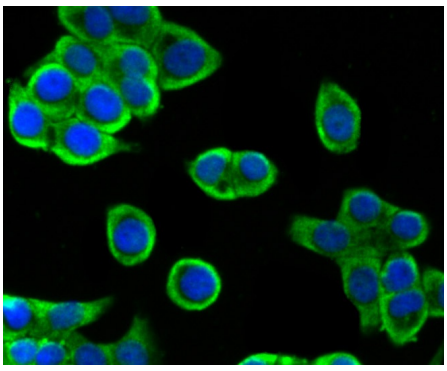

Fig4: ICC staining of villin1 in LOVO cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET7106-62, 1/500) for 1 hour at 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).

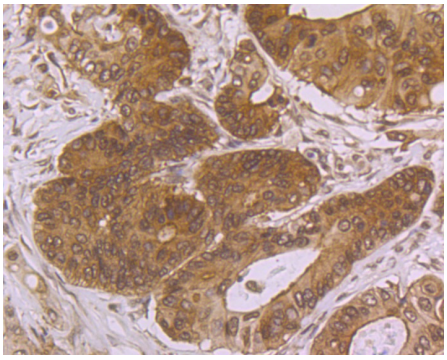

Fig5: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-villin1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET7106-62, 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.

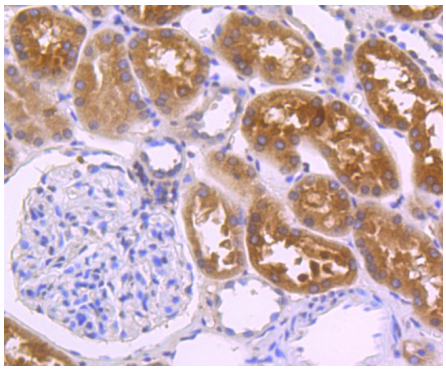

Fig6: Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-villin1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O 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.

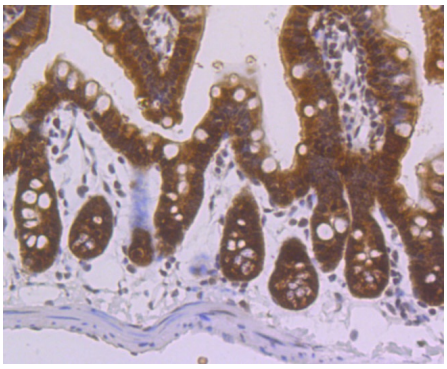

Fig7: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-villin1 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O 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.

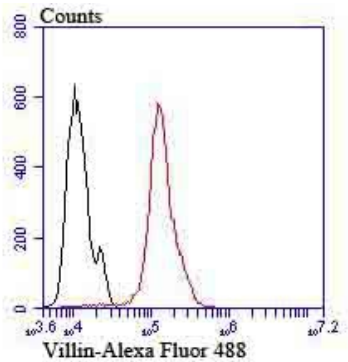

Fig8: Flow cytometric analysis of villin1 was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET7106-62, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

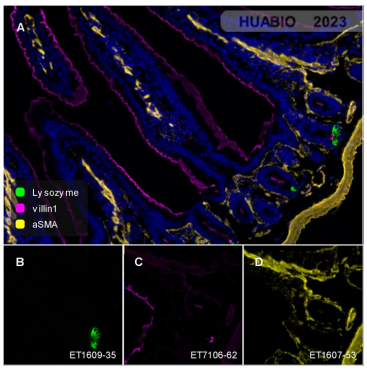

Fig9: Fluorescence multiplex immunohistochemical analysis of mouse small intestine (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-Lysozyme (ET1609-35, Green), anti-villin1 (ET7106-62, Magenta) and anti-aSMA (ET1607-53, Yellow) on mouse small intestine. HRP Conjugated 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℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

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).