Anti-Vimentin Antibody [A6-C1]

M1412-1

Product Type:	Mouse monoclonal IgG2b, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	A6-C1

Description: Vimentin is a type III intermediate filament (IF) protein that is expressed in mesenchymal cells. Vimentin plays a significant role in supporting and anchoring the position of the organelles in the cytosol. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. In essence, vimentin is responsible for maintaining cell shape, integrity of the cytoplasm, and stabilizing cytoskeletal interactions. Vimentin has been shown to eliminate toxic proteins in JUNQ and IPOD inclusion bodies in asymmetric division of asymmetric division of mammalian cell lines. It has been used as a sarcoma tumor marker to identify mesenchyme. Methylation of the vimentin gene has been established as a biomarker of colon cancer and this is being utilized in the development of fecal tests for colon cancer. High levels of DNA methylation in the promotor region have also been associated with markedly decreased survival in hormone positive breast cancers.

Immunogen: Synthetic peptide within Human Vimentin aa 1-50 / 466.

Positive control: HeLa cell lysate, C2C12 cell lysate, L6 cell lysate, HeLa, human kidney tissue.

Subcellular location: Cytoplasm, cytoskeleton, Nucleus matrix, Cell membrane.

Database links: SwissProt: P08670 Human | P20152 Mouse | P31000 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100-1:200
IHC-P	1:400

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% SodiumAzide.

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

Purity: Protein A affinity purified.

Images

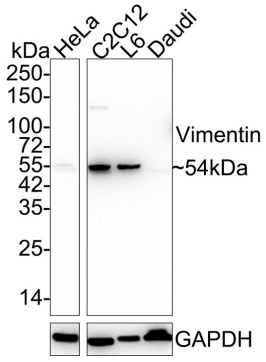

Fig1: Western blot analysis of Vimentin on different lysates with Mouse anti-Vimentin antibody (M1412-1) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: C2C12 cell lysate
Lane 3: L6 cell lysate
Lane 4: Daudi cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 24 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 (M1412-1) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

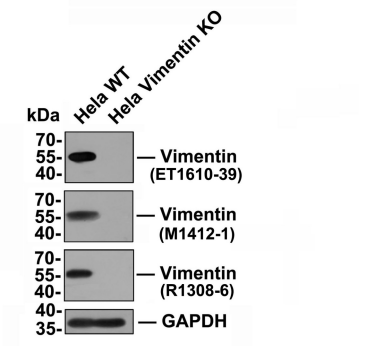

Fig2: All lanes: Western blot analysis of Vimentin with anti-Vimentin antibody (M1412-1) at 1:500 dilution.
Lane 1: Wild-type Hela whole cell lysate (10 µg).
Lane 2: Vimentin knockout Hela whole cell lysate (10 µg).

M1412-1 was shown to specifically react with Vimentin in wild-type Hela cells. No band was observed when Vimentin knockout sample was tested. Wild-type and Vimentin knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (M1412-1, 1:500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG-HRP Secondary Antibody (HA1006) at 1:100,000 dilution was used for 1 hour at room temperature.

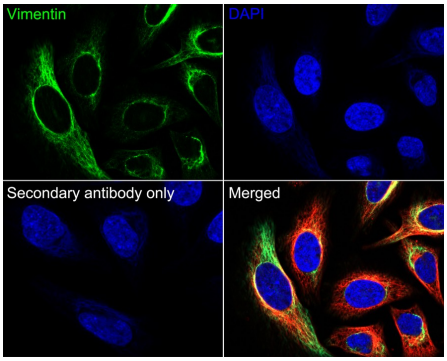

Fig3: Immunocytochemistry analysis of HeLa cells labeling Vimentin with Mouse anti-Vimentin antibody (M1412-1) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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 Mouse anti-Vimentin antibody (M1412-1) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

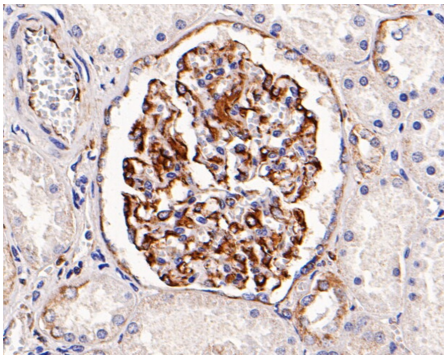

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-Vimentin antibody (M1412-1) at 1/400 dilution.

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 (M1412-1) at 1/400 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.

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

Background References

1. Ridge KM et al. Roles of vimentin in health and disease. Genes Dev. 2022 Apr
2. Kuburich NA et al. Vimentin and cytokeratin: Good alone, bad together. Semin Cancer Biol. 2022 Nov