Anti-Vimentin Antibody [SC60-05] - BSA and Azide free

HA750215

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	WB, IHC-P, IHC-Fr, IF-Tissue, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	SC60-05

Description: Vimentin (57 kDa) is the most ubiquituos intermediate filament protein and the first to be expressed during cell differentiation. All primitive cell types express vimentin but in most non-mesenchymal cells it is replaced by other intermediate filament proteins during differentiation. Vimentin is expressed in a wide variety of mesenchymal cell types: fibroblasts, endothelial cells etc., and in a number of other cell types derived from mesoderm, e.g., mesothelium and ovarian granulosa cells. Vimentin is present in many different neoplasms but is particulary expressed in those originated from mesenchymal cells. Sarcomas e.g., fibrosarcoma, malignt fibrous histiocytoma, angiosarcoma, and leio- and rhabdomyosarcoma, as well as lymphomas, malignant melanoma and schwannoma, are virtually always vimentin positive. Mesoderm derived carcinomas like renal cell carcinoma, adrenal cortical carcinoma and adenocarcinomas from endometrium and ovary usually express vimentin. Also thyroid carcinomas are vimentin positive. Any low differentiated or sarcomatoid carcinoma may express some vimentin. Vimentin is frequently included in the so-called primary panel (together with CD45, cytokeratin, and S-100 protein): Intense staining reaction for vimentin without coexpression of other intermediate filament proteins is strongly suggestive of a mesenchymal tumour or a malignant melanoma. However, in biopsies representing only a sarcomatoid part of renal cell carcinoma a.o. a strong positivity for vimentin without cytokeratin expression may be seen. Tumours like lymphomas and seminomas have the same intermediate filament profile, but the vimentin expression is usually weaker.

Immunogen: Synthetic peptide within C-terminal human Vimentin.

Positive control: HeLa cell lysate, HEK-293 cell lysate, Jurkat cell lysate, NIH/3T3 cell lysate, C6 cell lysate, C2C12 cell lysate, RAW264.7 cell lysate, C6, HeLa, L6, C2C12, human endometrial carcinoma tissue, mouse colon tissue, mouse cerebellum tissue, human breast carcinoma tissue, human colon tissue, human kidney tissue, mouse large intestine tissue.

Subcellular location: Cytoplasm.

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

Recommended Dilutions:

WB	1:20,000-1:50,000
IHC-P	1:5,000-1:10,000
IHC-Fr	1:1,000-1:2,000
IF-Tissue	1:1,000-1:2,000
IF-Cell	1:100-1:500
FC	1:1,000
IP	Use at an assay dependent concentration.

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

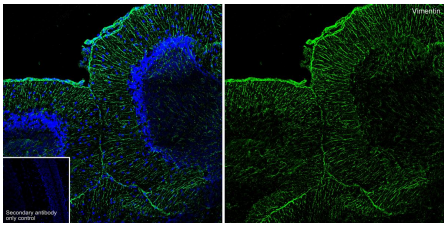

Fig1: Application: IHC-Fr

Species: Mouse

Site: Cerebellum

Sample: Frozen section

Antibody concentration: 1/2,000

Antigen retrieval: Not required

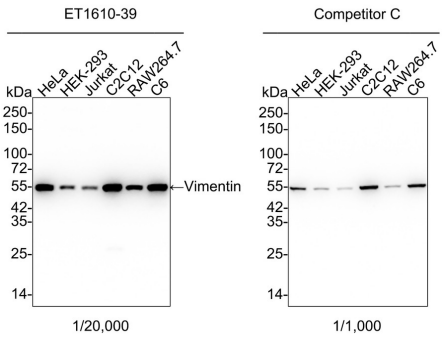

Fig2: Western blot analysis of Vimentin on different lysates with Rabbit anti-Vimentin antibody (HA750215) at 1/20,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: HEK-293 cell lysate (10 µg/Lane)
Lane 3: Jurkat cell lysate (10 µg/Lane)
Lane 4: C2C12 cell lysate (10 µg/Lane)
Lane 5: RAW264.7 cell lysate (10 µg/Lane)
Lane 6: C6 cell lysate (10 µg/Lane)

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

Exposure time: 14 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 (HA750215) at 1/20,000 dilution and competitor's antibody at 1/1,000 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.

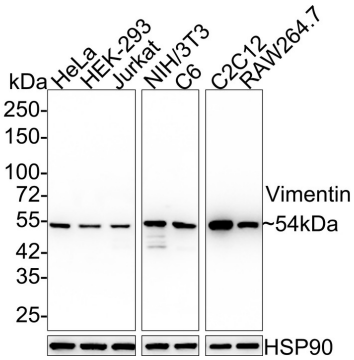

Fig3: Western blot analysis of Vimentin on different lysates with Rabbit anti-Vimentin antibody (HA750215) at 1/20,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: HEK-293 cell lysate (10 µg/Lane)
Lane 3: Jurkat cell lysate (10 µg/Lane)
Lane 4: NIH/3T3 cell lysate (10 µg/Lane)
Lane 5: C6 cell lysate (10 µg/Lane)
Lane 6: C2C12 cell lysate (10 µg/Lane)
Lane 7: RAW264.7 cell lysate (10 µg/Lane)

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

Exposure time: Lane 1-5: 3 seconds; Lane 6-7: 14 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 (HA750215) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

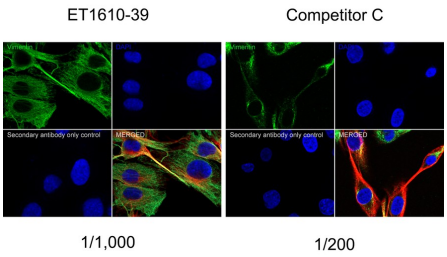

Fig4: Immunocytochemistry analysis of C6 cells labeling Vimentin with Rabbit anti-Vimentin antibody (HA750215) at 1/1,000 dilution and competitor's antibody at 1/200 dilution.

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 Rabbit anti-Vimentin antibody (HA750215) at 1/1,000 dilution and competitor's antibody at 1/200 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 (M1305-2, 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.

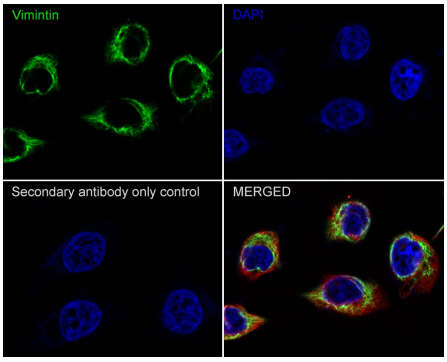

Fig5: Immunocytochemistry analysis of HeLa cells labeling Vimentin with Rabbit anti-Vimentin antibody (HA750215) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Vimentin antibody (HA750215) at 1/200 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, 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.

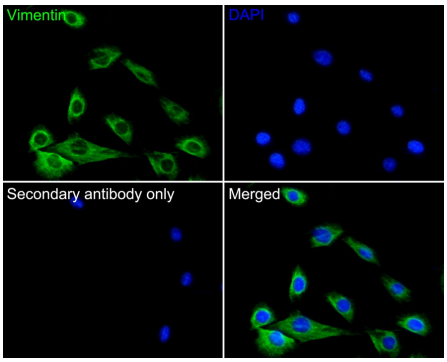

Fig6: Immunocytochemistry analysis of L6 cells labeling Vimentin with Rabbit anti-Vimentin antibody (HA750215) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Vimentin antibody (HA750215) at 1/50 dilution in 2% negative goat serum 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.

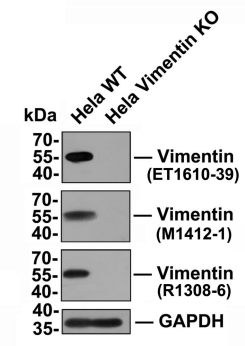

Fig7: All lanes: Western blot analysis of Vimentin with anti-Vimentin antibody (HA750215) at 1/5,000 dilution.
Lane 1: Wild-type Hela whole cell lysate (10 µg).
Lane 2: Vimentin knockout Hela whole cell lysate (10 µg).

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1610-39, 1/5,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1/300,000 dilution was used for 1 hour at room temperature.

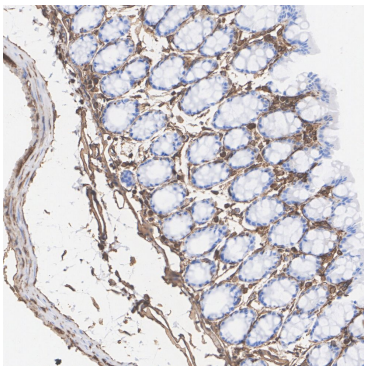

Fig8: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/5,000 dilution.

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 (HA750215) at 1/5,000 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.

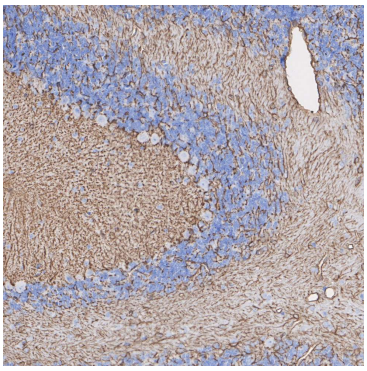

Fig9: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/5,000 dilution.

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 (HA750215) at 1/5,000 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.

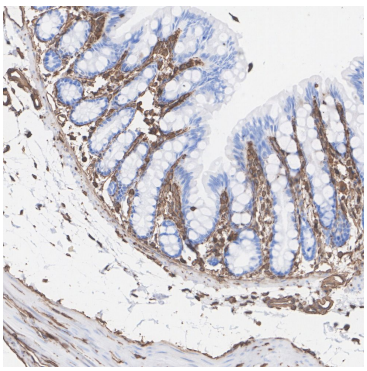

Fig10: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/5,000 dilution.

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 (HA750215) at 1/5,000 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.

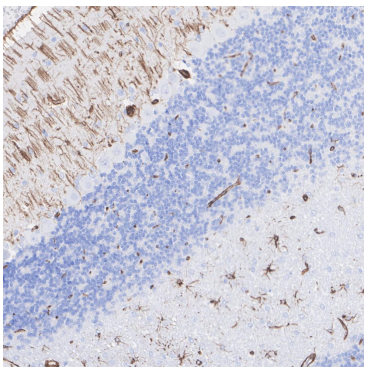

Fig11: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/5,000 dilution.

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 (HA750215) at 1/5,000 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.

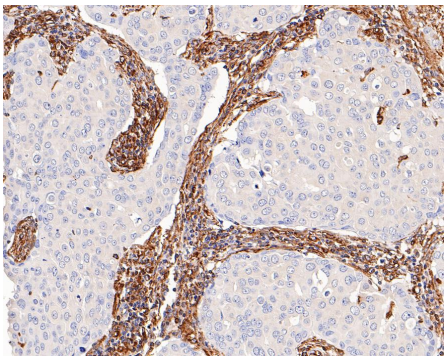

Fig12: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/10,000 dilution.

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 (HA750215) at 1/10,000 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.

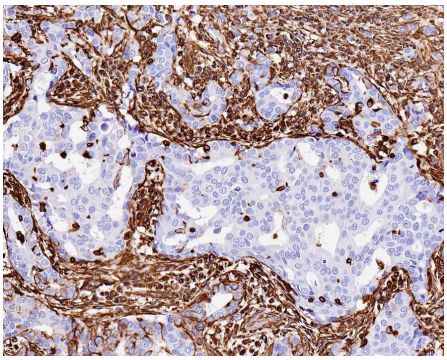

Fig13: Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/5,000 dilution.

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 (HA750215) at 1/5,000 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.

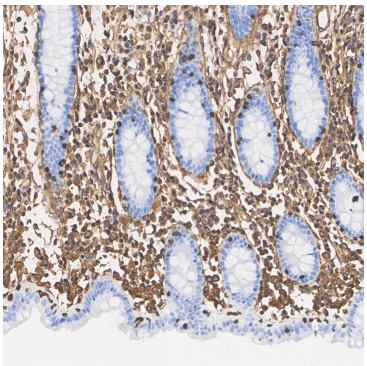

Fig14: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/5,000 dilution.

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 (HA750215) at 1/5,000 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.

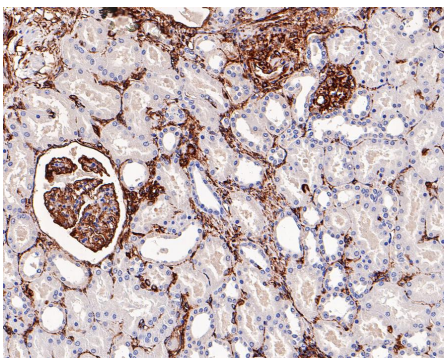

Fig15: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Vimentin antibody (HA750215) at 1/5,000 dilution.

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 (HA750215) at 1/5,000 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.

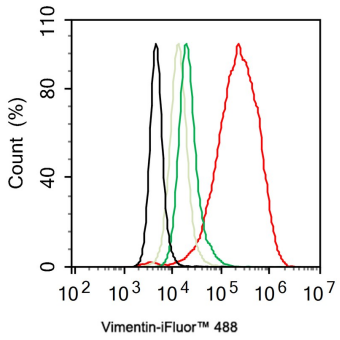

Fig16: Flow cytometric analysis of HeLa (positive, red) and MCF7 (negative, green) cells labeling Vimentin.

Cells were fixed by 4% formaldehyde and then permeabilized with ice-cold 90% methanol. Then stained with the primary antibody (HA750215) at 1/1,000 dilution, compared with Rabbit IgG Isotype Control (HeLa black, MCF7 light 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℃.

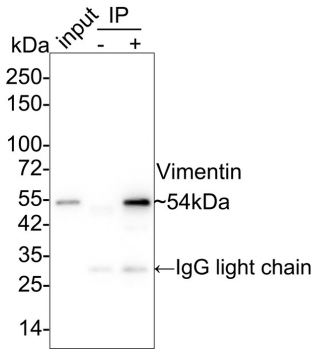

Fig17: Vimentin was immunoprecipitated in 0.2mg HeLa cell lysate with HA750215 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA750215 at 1/10,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: Rabbit IgG instead of HA750215 in HeLa cell lysate
Lane 3: HA750215 IP in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 5 seconds

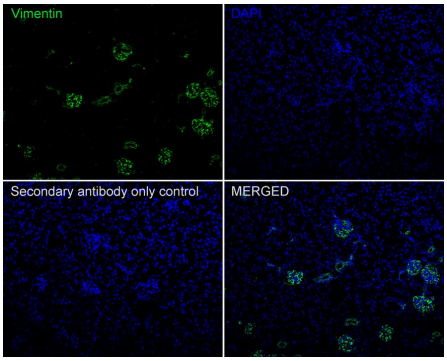

Fig18: Application: IF-tissue

Species: Mouse

Site: Kindey

Sample: Paraffin-embedded section

Antibody concentration: 1/1,000

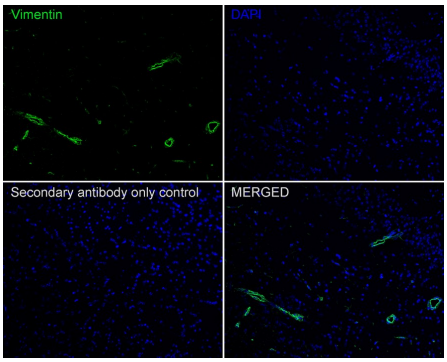

Fig19: Application: IF-tissue

Species: Mouse

Site: Cerebral cortex

Sample: Paraffin-embedded section

Antibody concentration: 1/1,000

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