

# Anti-Vimentin Antibody [SC60-05]

ET1610-39



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

**Description:** Vimentin (57 kDa) is the most ubiquitous 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 particularly expressed in those originated from mesenchymal cells. Sarcomas e.g., fibrosarcoma, malignant fibrous histiocytoma, angiosarcoma, and lei- 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 kidney tissue, human breast carcinoma tissue, human large intestine tissue, human kidney tissue, mouse large intestine tissue.

**Subcellular location:** Cytoplasm.

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

**Recommended Dilutions:**

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

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

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

**Purity:** Protein A affinity purified.

Hangzhou Huan Biotechnology Co., Ltd.

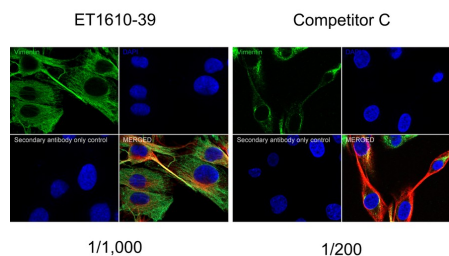
Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物  
HUABIO  
www.huabio.cn

## Images

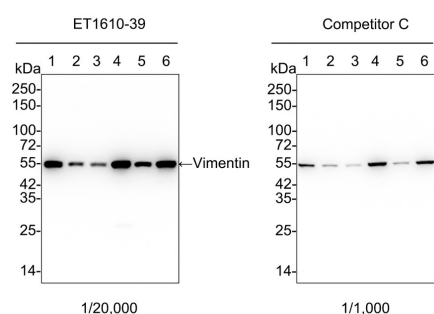


**Fig1:** Immunocytochemistry analysis of C6 cells labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39) at 1/1,000 dilution and competitor's antibody at 1/200 dilution.

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 (ET1610-39) at 1/1,000 dilution and competitor's antibody at 1/200 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

**Fig2:** Western blot analysis of Vimentin on different lysates with Rabbit anti-Vimentin antibody (ET1610-39) 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;

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 (ET1610-39) at 1/20,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

Hangzhou Huaan Biotechnology Co., Ltd.

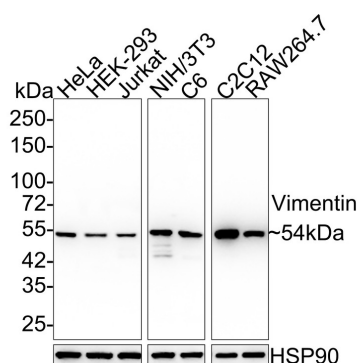
Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物  
 HUABIO  
 www.huabio.cn

**Fig3:** Western blot analysis of Vimentin on different lysates with Rabbit anti-Vimentin antibody (ET1610-39) 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

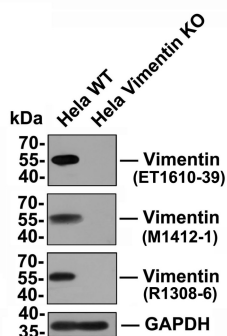
Exposure time: Lane 1-5: 3 seconds; Lane 6-7: 14 seconds;

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 (ET1610-39) 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:** All lanes: Western blot analysis of Vimentin with anti-Vimentin antibody (ET1610-39) at 1:500 dilution.

Lane 1: Wild-type Hela whole cell lysate (10 µg).  
 Lane 2: Vimentin knockout Hela whole cell lysate (10 µg).



ET1610-39 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 (ET1610-39, 1:500) 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.

Hangzhou Huaan Biotechnology Co., Ltd.

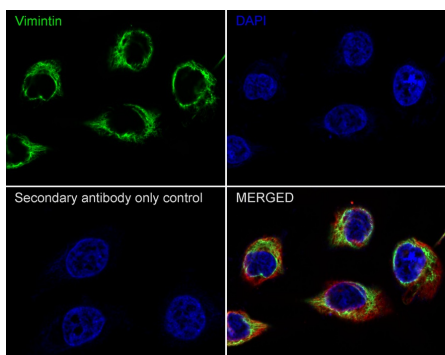
Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物  
 HUABIO  
 www.huabio.cn

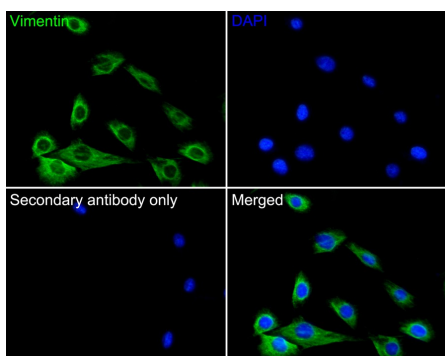
**Fig5:** Immunocytochemistry analysis of HeLa cells labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39) at 1/200 dilution.



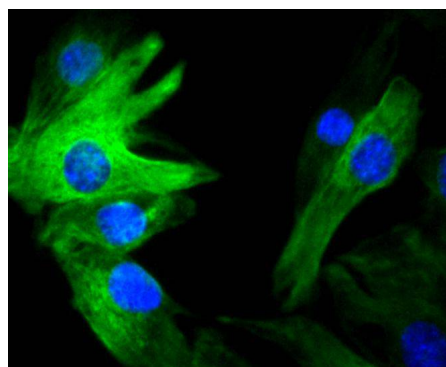
Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Vimentin antibody (ET1610-39) at 1/200 dilution in 2% negative goat serum overnight at 4 °C. 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 °C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

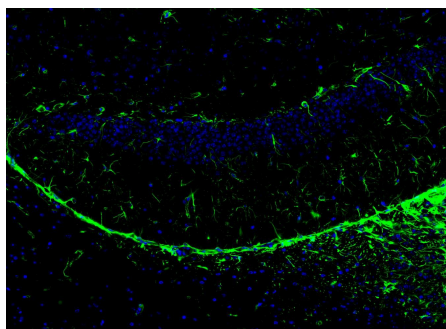
**Fig6:** Immunocytochemistry analysis of L6 cells labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39) at 1/50 dilution.



Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 °C, 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 (ET1610-39) at 1/50 dilution in 2% negative goat serum overnight at 4 °C. 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.

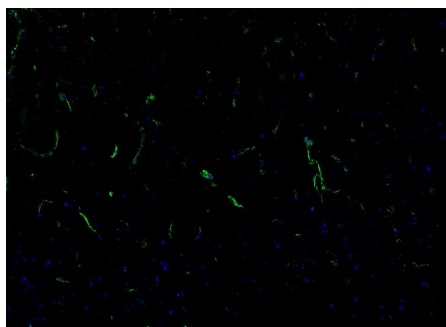


**Fig7:** ICC staining of Vimentin in C2C12 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-39, 1/50) for 1 hour 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).



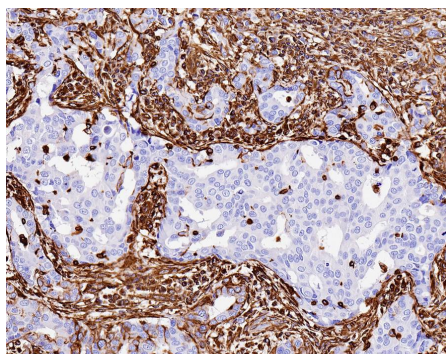
**Fig8:** Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-39, green) at 1/100 dilution overnight at 4°C, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.



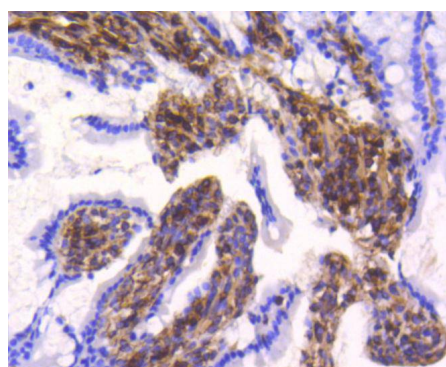
**Fig9:** Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-39, green) at 1/100 dilution overnight at 4°C, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

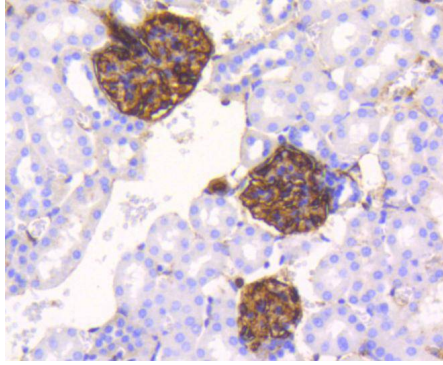


**Fig10:** Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue with Rabbit anti-Vimentin antibody (ET1610-39) at 1/1,000 dilution.

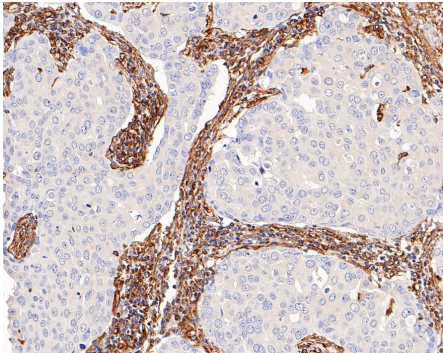
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1610-39) at 1/1,000 dilution for 1 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.



**Fig11:** Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-Vimentin 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1610-39, 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.

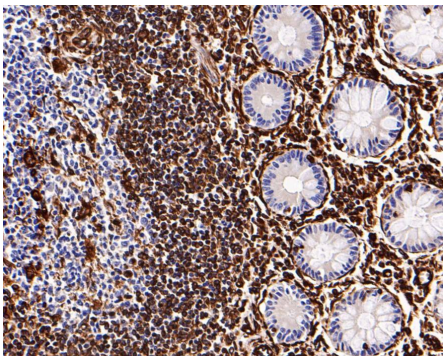


**Fig12:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue using anti-Vimentin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1610-39, 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.



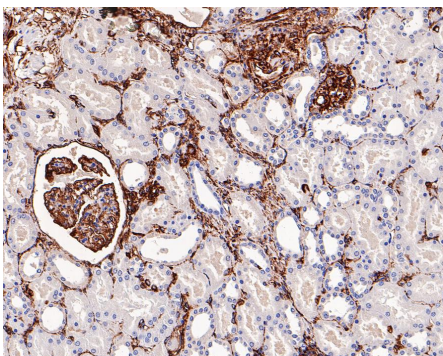
**Fig13:** Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Vimentin antibody (ET1610-39) at 1/10,000 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1610-39) 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.



**Fig14:** Immunohistochemical analysis of paraffin-embedded human large intestine tissue with Rabbit anti-Vimentin antibody (ET1610-39) at 1/2,000 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1610-39) at 1/2,000 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 (ET1610-39) 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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1610-39) 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.

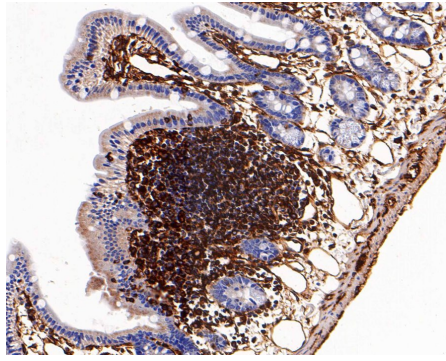
Hangzhou Huan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

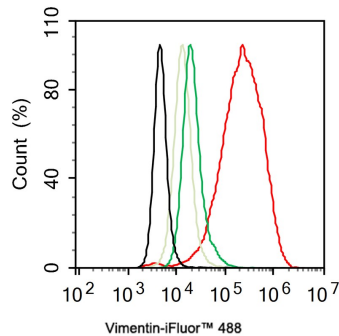
Service mail:support@huabio.cn

华安生物  
HUABIO  
www.huabio.cn



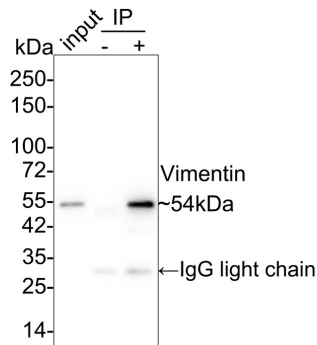
**Fig16:** Immunohistochemical analysis of paraffin-embedded mouse large intestine tissue with Rabbit anti-Vimentin antibody (ET1610-39) at 1/500 dilution.

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<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1610-39) at 1/500 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.



**Fig17:** Flow cytometric analysis of HeLa (positive, red) and MCF7 (negative, green) cells labeling Vimentin.

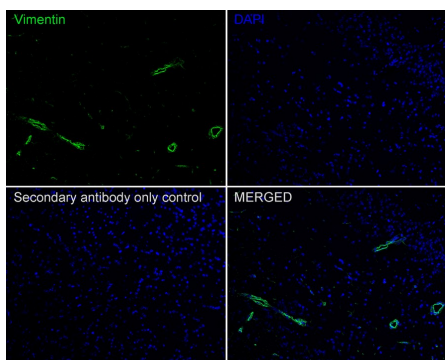
Cells were fixed by 4% formaldehyde and then permeabilized with ice-cold 90% methanol. Then stained with the primary antibody (ET1610-39) at 1/1,000 dilution, compared with Rabbit IgG Isotype Control (HeLa black, MCF7 light green). After incubation of the primary antibody at +4°C for an hour, the cells 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.



**Fig18:** Vimentin was immunoprecipitated in 0.2mg HeLa cell lysate with ET1610-39 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1610-39 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.

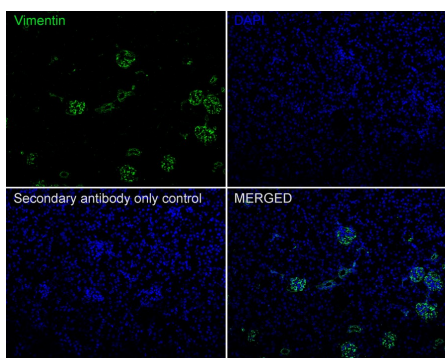
Lane 1: HeLa cell lysate (input)  
Lane 2: Rabbit IgG instead of ET1610-39 in HeLa cell lysate  
Lane 3: ET1610-39 IP in HeLa cell lysate

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



**Fig19:** Immunofluorescence analysis of paraffin-embedded mouse brain tissue labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39) 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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-39, green) at 1/1,000 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).



**Fig20:** Immunofluorescence analysis of paraffin-embedded mouse kidney tissue labeling Vimentin with Rabbit anti-Vimentin antibody (ET1610-39) 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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1610-39, green) at 1/1,000 dilution overnight at 4 °C, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

**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

Hangzhou Huan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

Service mail:support@huabio.cn

华安生物  
HUABIO  
www.huabio.cn