# Anti-Vimentin Antibody [D4-B11]

## EM0401

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|---|---|
| **Product Type:** | Mouse monoclonal IgG1, primary antibodies |
| **Species reactivity:** | Human, Mouse, Rat |
| **Applications:** | WB, IF-Cell, IHC-P, FC, IF-Tissue, IHC-Fr, IP |
| **Molecular Wt:** | Predicted band size: 54 kDa |
| **Clone number:** | D4-B11 |

**Description:** Vimentin is a structural protein that in humans is encoded by the VIM gene. Its name comes from the Latin vimentum which refers to an array of flexible rods. Vimentin is a type III intermediate filament (IF) protein that is expressed in mesenchymal cells. IF proteins are found in all animal cells[6] as well as bacteria. Intermediate filaments, along with tubulin-based microtubules and actin-based microfilaments, comprises the cytoskeleton. All IF proteins are expressed in a highly developmentally-regulated fashion; vimentin is the major cytoskeletal component of mesenchymal cells. Because of this, vimentin is often used as a marker of mesenchymally-derived cells or cells undergoing an epithelial-to-mesenchymal transition (EMT) during both normal development and metastatic progression. 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.

**Immunogen:** Synthetic peptide within C-terminal human Vimentin.

**Positive control:** HeLa cell lysate, C2C12 cell lysate, C6 cell lysate, NIH/3T3 cell lysate, Mouse embryonic stem cell lysate, Hela, human kidney tissue, human colon carcinoma tissue, human stomach carcinoma tissue, human tonsil tissue, human skin tissue, human liver tissue, human appendix tissue, human endometrium tissue.

**Subcellular location:** Cytoplasm

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

**Recommended Dilutions:**

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| **WB** | 1:2,000-1:5,000 |
| **IF-Cell** | 1:200 |
| **IHC-P** | 1:200 |
| **FC** | 1:500-1:1,000 |
| **IF-Tissue** | 200-400 |
| **IHC-Fr** | 1:100 |
| **IP** | Use at an assay dependent concentration |

**Storage Buffer:** 1*PBS (pH7.4), 0.2% 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

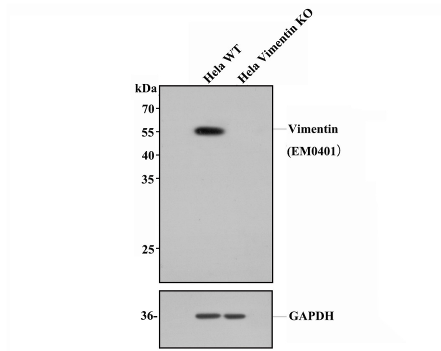


**Fig1:** All lanes: Western blot analysis of Vimentin with anti-Vimentin antibody [D4-B11] (EM0401) at 1:500 dilution.
Lane 1: Wild-type Hela whole cell lysate (20 µg).
Lane 2: Vimentin knockout Hela whole cell lysate (20 µg).

EM0401 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 (EM0401, 1/500) and Loading control antibody (Rabbit anti-GAPDH , ET1601-4, 1/10,000) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Mouse IgG-HRP Secondary Antibody (HA1006) at 1:20,000 dilution was used for 1 hour at room temperature.

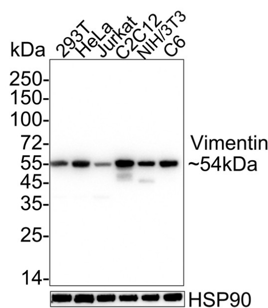


**Fig2:** Western blot analysis of Vimentin on different lysates with Rabbit anti-Vimentin antibody (EM0401) at 1/1,000 dilution.

Lane 1: 293T cell lysate
Lane 2: HeLa cell lysate
Lane 3: Jurkat cell lysate
Lane 4: C2C12 cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: C6 cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time:15 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 (EM0401) at 1/1,000 dilution was 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.

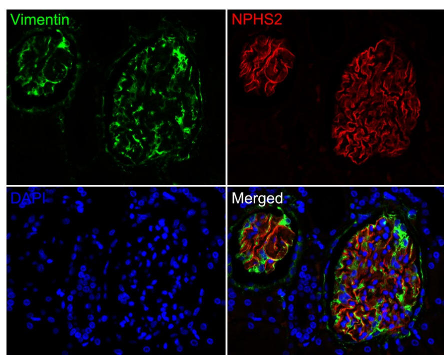


**Fig3:** Immunofluorescence analysis of paraffin-embedded human kidney tissue labeling Vimentin (EM0401) and NPHS2 (ET7107-34).

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 antibodies Vimentin (EM0401, green) at 1/400 dilution and NPHS2 (ET7107-34, red) at 1/100 dilution overnight at 4 ℃, washed with PBS.

iFluor™ 488 conjugate-Goat anti-Mouse IgG (HA1125) and iFluor™ 594 conjugate-Goat anti-Rabbit IgG (HA1122) were used as the secondary antibodies at 1/1,000 dilution. DAPI was used as nuclear counterstain.

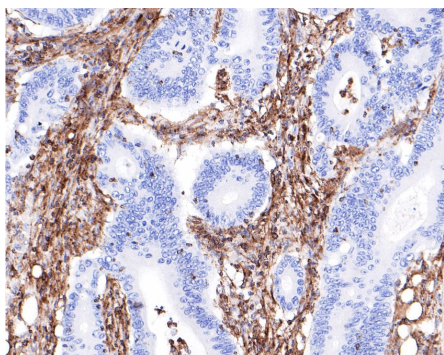

**Fig4:** Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-Vimentin antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401, 1/800) 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.

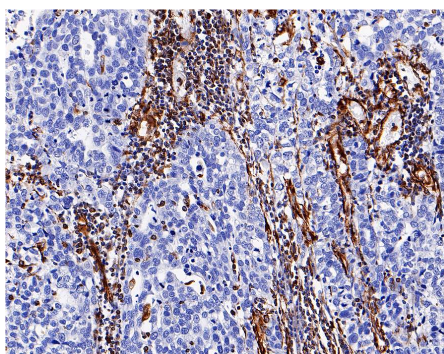

**Fig5:** Immunohistochemical analysis of paraffin-embedded human stomach carcinoma tissue with Mouse anti-Vimentin antibody (EM0401) at 1/2,000 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401) 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.

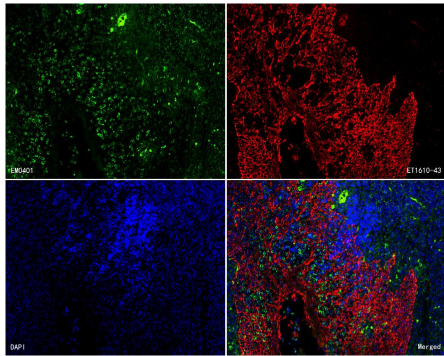


**Fig6:** Immunofluorescence analysis of paraffin-embedded human tonsil tissue labeling Vimentin (EM0401) at 1/200 dilution and Cytokeratin 5 (ET1610-43) at 1/200 dilution.
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Vimentin (EM0401, green) at 1/200 dilution and Cytokeratin 5 (ET1610-43, red) at 1/200 dilution at +4℃ overnight, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Mouse IgG and Alexa Fluor®594 conjugate-Goat anti-Rabbit IgG were used as the secondary antibodies at 1/1,000 dilution. DAPI was used as nuclear counterstain.

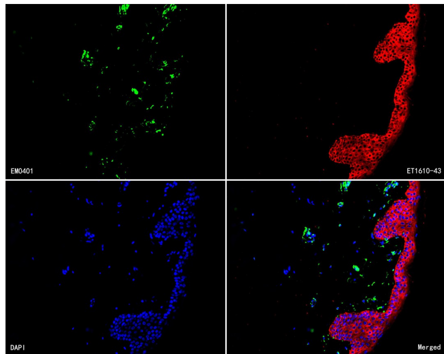


**Fig7:** Immunofluorescence analysis of paraffin-embedded human skin tissue labeling Vimentin (EM0401) at 1/200 dilution and Cytokeratin 5 (ET1610-43) at 1/200 dilution.
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Vimentin (EM0401, green) at 1/200 dilution and Cytokeratin 5 (ET1610-43, red) at 1/200 dilution at +4℃ overnight, washed with PBS. Alexa Fluor®488 conjugate-Goat anti-Mouse IgG and Alexa Fluor®594 conjugate-Goat anti-Rabbit IgG were used as the secondary antibodies at 1/1,000 dilution. DAPI was used as nuclear counterstain.

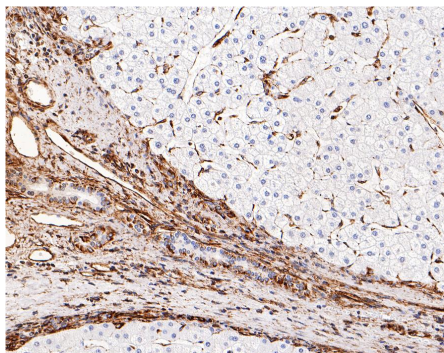

**Fig8:** Immunohistochemical analysis of paraffin-embedded human liver tissue with Mouse anti-Vimentin antibody (EM0401) at 1/2,000 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401) 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.

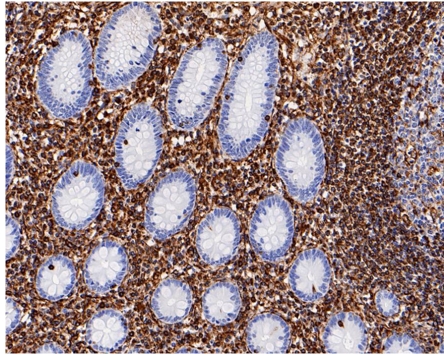

**Fig9:** Immunohistochemical analysis of paraffin-embedded human appendix tissue with Mouse anti-Vimentin antibody (EM0401) at 1/500 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401) 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.

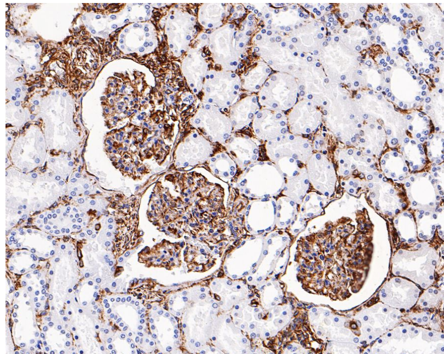

**Fig10:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Mouse anti-Vimentin antibody (EM0401) at 1/2,000 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401) 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.

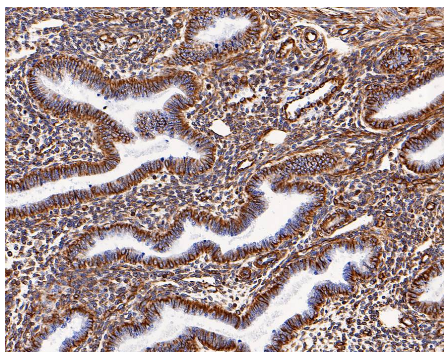

**Fig11:** Immunohistochemical analysis of paraffin-embedded human endometrium tissue with Mouse anti-Vimentin antibody (EM0401) at 1/500 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401) 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.

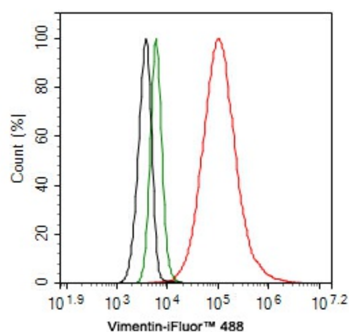


**Fig12:** Flow cytometric analysis of Hela cells labeling Vimentin.

Cells were fixed and permeabilized.Then stained with the primary antibody (EM0401, 1ug/ml) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

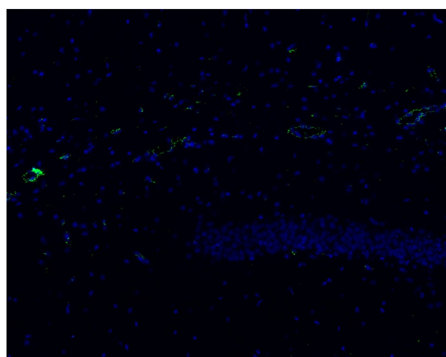

**Fig13:** Immunofluorescence analysis of frozen mouse hippocampus tissue labeling Vimentin with Rabbit anti-Vimentin antibody (EM0401).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (EM0401, green) at 1/100 dilution overnight at 4℃, 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.

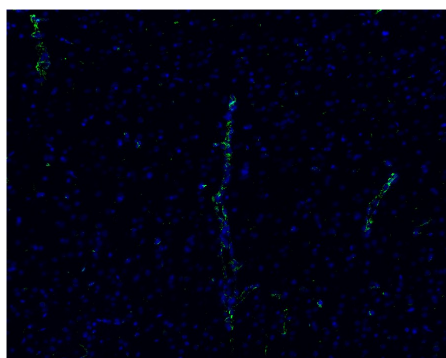

**Fig14:** Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling Vimentin with Rabbit anti-Vimentin antibody (EM0401).
The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (EM0401, green) at 1/100 dilution overnight at 4℃, 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.

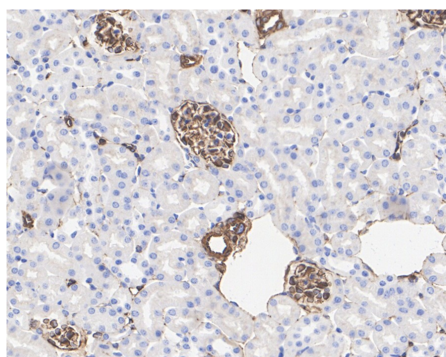

**Fig15:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Vimentin antibody (EM0401) at 1/1,000 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401) 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.

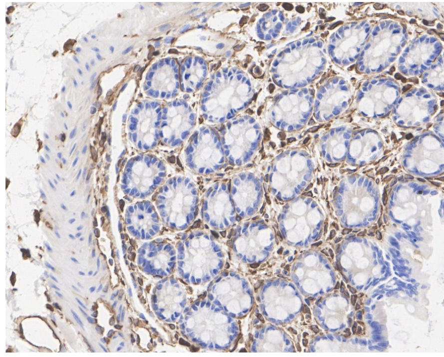

**Fig16:** Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Vimentin antibody (EM0401) at 1/1,000 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (EM0401) 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.

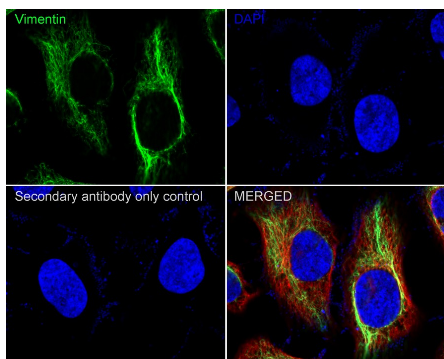


**Fig17:** Immunocytochemistry analysis of HeLa cells labeling Vimentin with Rabbit anti-Vimentin antibody (EM0401) at 1/100 dilution.

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 Rabbit anti-Vimentin antibody (EM0401) at 1/100 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.

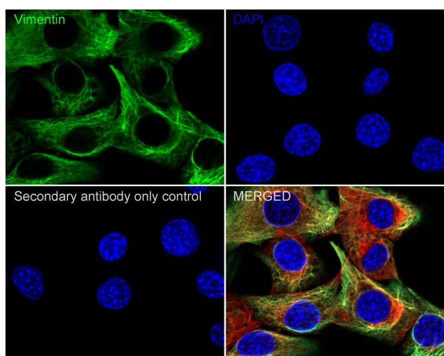


**Fig18:** Immunocytochemistry analysis of C2C12 cells labeling Vimentin with Rabbit anti-Vimentin antibody (EM0401) at 1/100 dilution.

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 Rabbit anti-Vimentin antibody (EM0401) at 1/100 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.

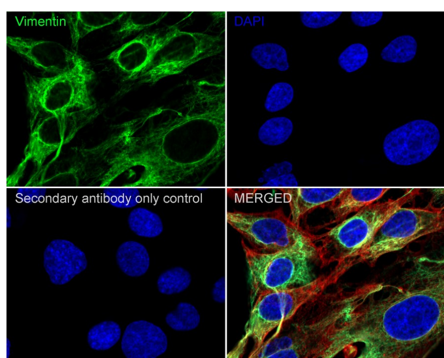


**Fig19:** Immunocytochemistry analysis of C6 cells labeling Vimentin with Rabbit anti-Vimentin antibody (EM0401) at 1/100 dilution.

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 Rabbit anti-Vimentin antibody (EM0401) at 1/100 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.

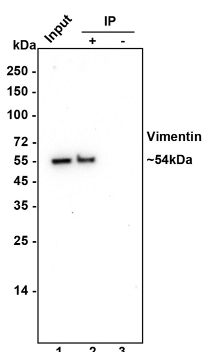


**Fig20:** Vimentin was immunoprecipitated in 0.2mg HeLa cell lysate with EM0401 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using EM0401 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: EM0401 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of EM0401 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 mintues

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

## Background References

1. "Aurora-B regulates the cleavage furrow-specific vimentin phosphorylation in the cytokinetic process." Goto H., Yasui Y., Kawajiri A., Nigg E.A., Terada Y., Tatsuka M., Nagata K., Inagaki M. J. Biol. Chem. 278:8526-8530(2003)

2. "Specific in vivo phosphorylation sites determine the assembly dynamics of vimentin intermediate filaments." Eriksson J.E., He T., Trejo-Skalli A.V., Harmala-Brasken A.-S., Hellman J., Chou Y.-H., Goldman R.D. J. Cell Sci. 117:919-932(2004)

3. "The cellular distribution of serotonin transporter is impeded on serotonin-altered vimentin network." Ahmed B.A., Bukhari I.A., Jeffus B.C., Harney J.T., Thyparambil S., Ziu E., Fraer M., Rusch N.J., Zimniak P., Lupashin V., Tang D., Kilic F. PLoS ONE 4:E4730-E4730(2009)