Anti-Phospho-Vimentin (S39) Antibody [JE43-26] HA721442

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell
Molecular Wt:	Predicted band size: 54 kDa
Clone number:	JE43-26

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. IF proteins are found in all animal cells 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.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Ser39 of human vimentin protein.

Positive control: HeLa treated with 100nM Calyculin A for 30 minutes whole cell lysate, HeLa treated with 100nM Calyculin A for 30 minutes, NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate, C6 treated with 100nM Calyculin A for 30 minutes cell lysate, NIH/3T3 treated with 100nM Calyculin A for 30 minutes.

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

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

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100

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℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

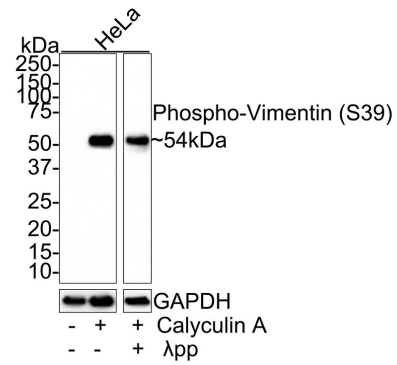

Fig1: Western blot analysis of Phospho-Vimentin (S39) on different lysates with Rabbit anti-Phospho-Vimentin (S39) antibody (HA721442) at 1/1,000 dilution.

Lane 1: HeLa whole cell lysate
Lane 2: HeLa treated with 100nM Calyculin A for 30 minutes whole cell lysate
Lane 3: HeLa treated with 100nM Calyculin A for 30 minutes whole cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 10 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 (HA721442) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

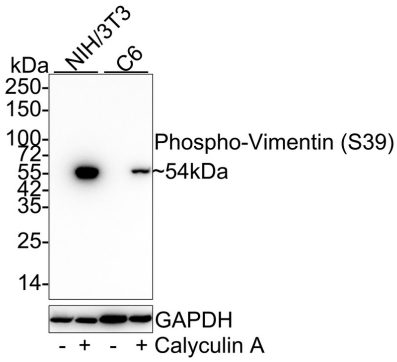

Fig2: Western blot analysis of Phospho-Vimentin (S39) on different lysates with Rabbit anti-Phospho-Vimentin (S39) antibody (HA721442) at 1/1,000 dilution.

Lane 1: NIH/3T3 cell lysate
Lane 2: NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 3: C6 cell lysate
Lane 4: C6 treated with 100nM Calyculin A for 30 minutes cell lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 30 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 (HA721442) at 1/1,000 dilution was used in 5%

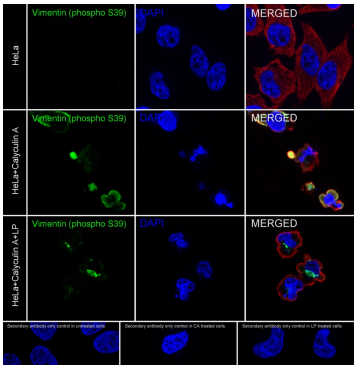

Fig3: Immunocytochemistry analysis of HeLa cells treated with or without 100nM Calyculin A for 30 minutes, then treated with λpp for 1 hour labeling Phospho-Vimentin (S39) with Rabbit anti-Phospho-Vimentin (S39) antibody (HA721442) at 1/100 dilution.

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-Phospho-Vimentin (S39) antibody (HA721442) at 1/100 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.

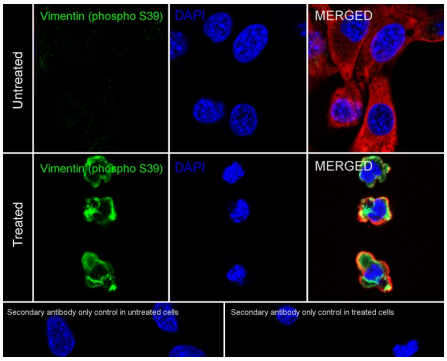

Fig4: Immunocytochemistry analysis of NIH/3T3 cells treated with or without 100nM Calyculin A for 30 minutes labeling Phospho-Vimentin (S39) with Rabbit anti-Phospho-Vimentin (S39) antibody (HA721442) at 1/100 dilution.

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-Phospho-Vimentin (S39) antibody (HA721442) 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.

Background References

1. Ridge KM et al. Roles of vimentin in health and disease. Genes Dev. 2022 Apr
2. Paulin D et al. Vimentin: Regulation and pathogenesis. Biochimie. 2022 Jun