

Product datasheet

Anti-Vimentin (phospho S56) antibody [EPR21084] ab217673

Recombinant RabMAb

[8 References](#) [6 Images](#)

Overview

Product name	Anti-Vimentin (phospho S56) antibody [EPR21084]
Description	Rabbit monoclonal [EPR21084] to Vimentin (phospho S56)
Host species	Rabbit
Tested applications	Suitable for: WB, Dot blot, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa treated with 100 ng/ml nocodazole (ab120630) for 18 hours, whole cell lysate. ICC/IF: HeLa cells. IP: HeLa treated with 100 ng/ml nocodazole (ab120630) for 18 hours, whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number EPR21084
Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab217673 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. Detects a band of approximately 57 kDa (predicted molecular weight: 54 kDa).
Dot blot		1/1000.
ICC/IF		1/1000.
IP		1/30.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target

Function Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally.
Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2.

Tissue specificity Highly expressed in fibroblasts, some expression in T- and B-lymphocytes, and little or no expression in Burkitt's lymphoma cell lines. Expressed in many hormone-independent mammary carcinoma cell lines.

Involvement in disease Cataract 30

Sequence similarities Belongs to the intermediate filament family.

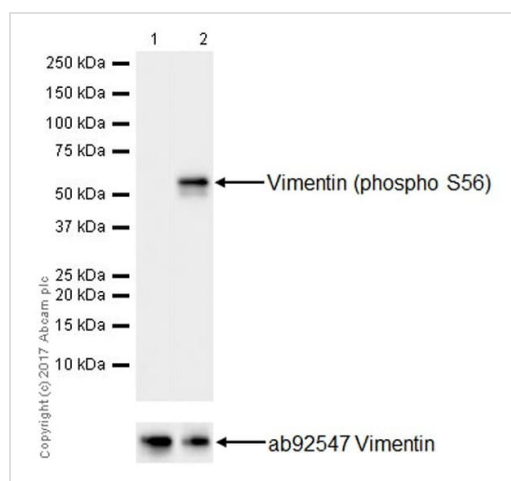
Domain The central alpha-helical coiled-coil rod region mediates elementary homodimerization. The [IL]-x-C-x-x-[DE] motif is a proposed target motif for cysteine S-nitrosylation mediated by the iNOS-S100A8/A9 transnitrosylase complex.

Post-translational modifications Filament disassembly during mitosis is promoted by phosphorylation at Ser-55 as well as by nestin (By similarity). One of the most prominent phosphoproteins in various cells of mesenchymal origin. Phosphorylation is enhanced during cell division, at which time vimentin filaments are significantly reorganized. Phosphorylation by PKN1 inhibits the formation of filaments. Phosphorylated at Ser-56 by CDK5 during neutrophil secretion in the cytoplasm. Phosphorylated by STK33.
O-glycosylated during cytokinesis at sites identical or close to phosphorylation sites, this interferes with the phosphorylation status.
S-nitrosylation is induced by interferon-gamma and oxidatively-modified low-density lipoprotein (LDL(ox)) possibly implicating the iNOS-S100A8/9 transnitrosylase complex.

Cellular localization Cytoplasm.

Form Vimentin is found in connective tissue and in the cytoskeleton.

Images



Western blot - Anti-Vimentin (phospho S56) antibody [EPR21084] (ab217673)

All lanes : Anti-Vimentin (phospho S56) antibody [EPR21084] (ab217673) at 1/1000 dilution

Lane 1 : Untreated HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) treated with 100 ng/ml nocodazole ([ab120630](#)) for 18 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

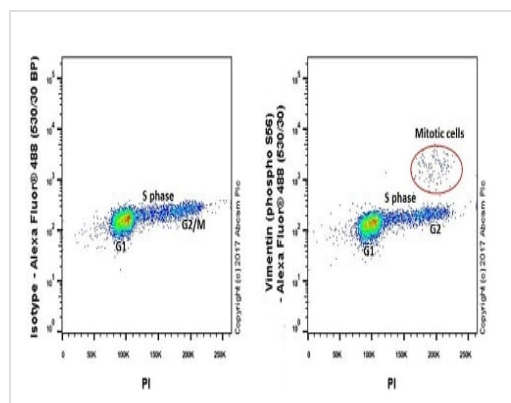
Developed using the ECL technique.

Predicted band size: 54 kDa

Observed band size: 57 kDa

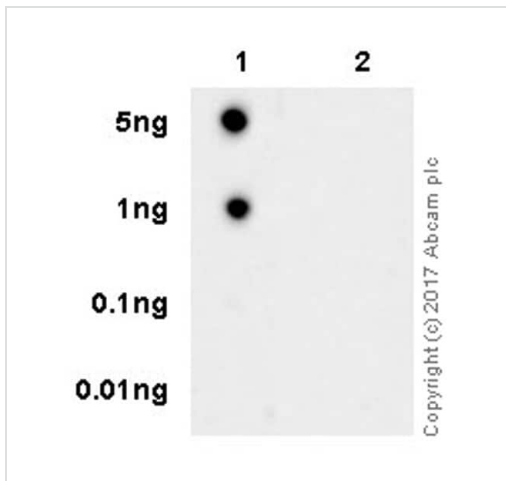
Exposure time: 23 seconds

Blocking/Dilution buffer: 5% NFDm/TBST.



Flow Cytometry (Intracellular) - Anti-Vimentin (phospho S56) antibody [EPR21084] (ab217673)

Ab217673 staining Vimentin (phospho S56) in HeLa (human cervix adenocarcinoma epithelial cell) cells by intracellular flow cytometry. Cells were fixed using 80% Methanol and permeabilized with 0.1% Tween-20. The sample was incubated with primary antibody at 1/500 dilution. An Alexa Fluor®488 Goat anti-rabbit IgG ([ab150077](#)) was used as a secondary antibody. Rabbit monoclonal IgG ([ab172730](#)) was used as an isotype control (left). Cells were pre-treated with 20 µg/ml RNase A for 30 minutes to eliminate the non-specific binding between RNA and PI (Propidium iodide). Vimentin (phospho S56) is highly expressed in mitotic cells. (PMID: 16260496)



Dot Blot - Anti-Vimentin (phospho S56) antibody [EPR21084] (ab217673)

Dot blot analysis of Vimentin (phospho S56) labeled with ab217673 at 1/1000 dilution.

Lane 1: Vimentin (phospho S56) peptide.

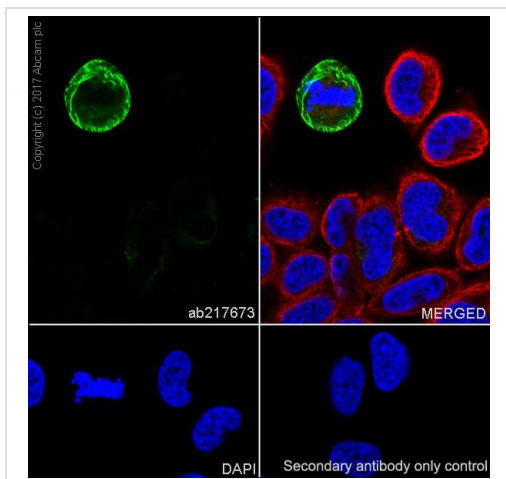
Lane 2: Vimentin non-phospho peptide.

Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution was used as secondary antibody.

Blocking and dilution buffer: 5% NFD/MTBST.

Exposure time: 1 minute.

The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument.

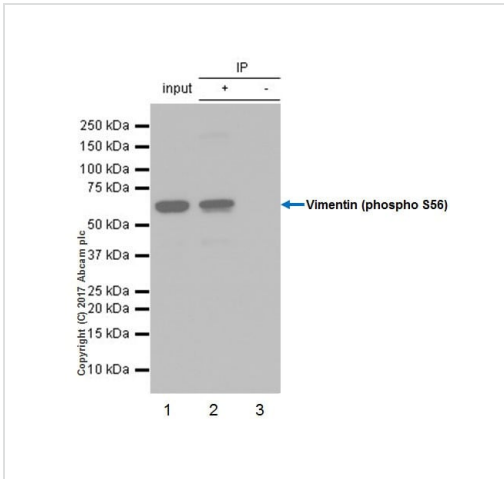


Immunocytochemistry/ Immunofluorescence - Anti-Vimentin (phospho S56) antibody [EPR21084] (ab217673)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Vimentin (phospho S56) with ab217673 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution (green). Confocal image showing strong positive staining in HeLa cells in M phase.

The nuclear counterstain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) ([ab195889](#)) at 1/200 dilution (red).

Secondary antibody only control: PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-Vimentin (phospho S56) antibody [EPR21084] (ab217673)

Vimentin (phospho S56) was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) treated with 100 ng/ml nocodazole (**ab120630**) for 18 hours, whole cell lysate with ab217673 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab217673 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.





Lane 1: HeLa treated with 100 ng/ml nocodazole (**ab120630**) for 18 hours, whole cell lysate 10 µg (Input).

Lane 2: ab217673 IP in HeLa treated with 100 ng/ml nocodazole (**ab120630**) for 18 hours, whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab217673 in HeLa treated with 100 ng/ml nocodazole (**ab120630**) for 18 hours, whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDN/TBST.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

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