# abcam

### Product datasheet

## Anti-Vimentin antibody - Cytoskeleton Marker ab45939



★★★★★ 17 Abreviews 182 References 画像数 13

製品の概要

製品名 Anti-Vimentin antibody - Cytoskeleton Marker

製品の詳細 Rabbit polyclonal to Vimentin - Cytoskeleton Marker

由来種 Rabbit

特異性 Replenishment batches of our polyclonal antibody, ab45939 are tested in WB. Previous batches

were additionally validated in Flow Cyt, ICC/IF and IHC-P. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative

recombinant antibody, ab92547.

アプリケーション 適用あり: IHC-P, WB, ICC/IF, Flow Cyt (Intra)

**種交差性 交差種:** Mouse, Rat, Human

交差が予測される動物種: Sheep, Rabbit, Horse, Chicken, Cow, Pig, Chimpanzee

Synthetic peptide conjugated to KLH derived from within residues 400 to the C-terminus of

Human Vimentin.Immunogen の所有権に関して(Peptide available as <u>ab46156</u>)

**特記事項**The Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

製品の特性

免疫原

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

**バッファー** pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

1

agent. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help.

精製度 Immunogen affinity purified

**ポリ/モノ** ポリクローナル

アイソタイプ IgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab45939の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC-P	★★★★☆(9)	Use a concentration of 0.1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	<b>★★★☆☆(2)</b>	Use a concentration of 1 µg/ml. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).
ICC/IF	<b>★★★★★</b> (2)	Use a concentration of 1 µg/ml.
Flow Cyt (Intra)		Use 0.01-0.1µg for 10 <sup>6</sup> cells.

#### ターゲット情報

機能 Vimentins are class-Ill 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.

組織特異性 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.

**関連疾患** Cataract 30

**配列類似性** Belongs to the intermediate filament family.

ドメイン 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.

翻訳後修飾 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.

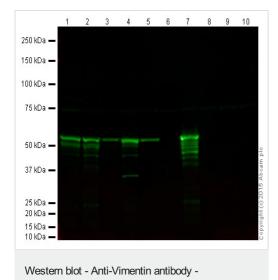
 $S-nitrosylation\ is\ induced\ by\ interferon-gamma\ and\ oxidatively-modified\ low-densitity\ lipoprotein$ 

(LDL(ox)) possibly implicating the iNOS-S100A8/9 transnitrosylase complex.

#### 製品の状態

Vimentin is found in connective tissue and in the cytoskeleton.

#### 画像



Cytoskeleton Marker (ab45939)

**All lanes :** Anti-Vimentin antibody - Cytoskeleton Marker (ab45939) at 1 μg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lane 3 : Jurkat (Human T cell lymphoblast-like cell line) Whole cell lysate

Lane 4: A549 (Human lung adenocarcinoma epithelial cell line)
Whole cell lysate

Lane 5: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

Lane 6 : PC12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate

Lane 7 : HUVEC (Human umbilical vein endothelial cell line) Whole cell lysate

**Lane 8**: A431 (Human epithelial carcinoma cell line) Whole cell lysate

Lane 9: Daudi (Human B lymphoblast) Whole cell lysate

Lane 10 : Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

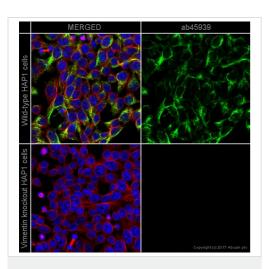
All lanes: IRDye® 800CW Goat Anti-Rabbit at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 54 kDa **Observed band size:** 53 kDa

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes.

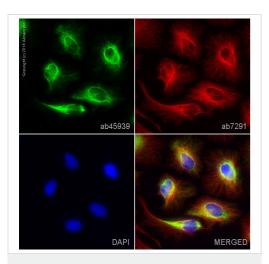
The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab45939 overnight at 4°C. The membrane was then blocked for an hour using LI-COR® blocking buffer before being incubated with ab92547 overnight at 4°C. Antibody binding was detected using the IRDye® 800CW Goat Anti-Rabbit secondary at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Odyssey® CLx Imaging System.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

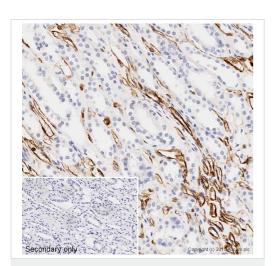
ab45939 staining Vimentin in wild-type HAP1 cells (top panel) and Vimentin knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab45939 at 1µg/ml dilution and ab195889 at 1/250 dilution (shown in pseudo-color red) overnight at +4°C. The cells were then incubated with ab150081 (Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)) at 1/1000 dilution for 1 hour. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

ab45939 staining Vimentin in HeLa cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab45939 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control, at 1/1000 dilution. Cells were then incubated with ab150120, Goat polyclonal Secondary Antibody to Mouse at 1/1000 dilution (shown in pseudocolour red) and ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG at 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

IHC image of ab45939 staining Vimentin in human kidney formalin fixed paraffin embedded tissue sections\*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab45939, 0.1µg/ml working concentration, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

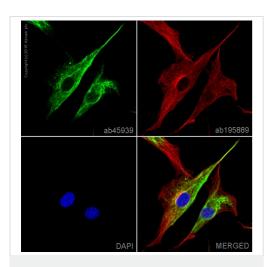
\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

ab45939 ab195889

Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

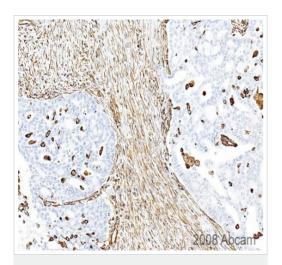
ab45939 staining Vimentin in SV40LT-SMC cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab45939 at 1µg/ml (shown in green) and <a href="mailto:ab195889">ab195889</a>, Mouse monoclonal [DM1A] to alpha Tubulin - Microtubule Marker (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

ab45939 staining Vimentin in NIH3T3 cells. The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab45939 at 1µg/ml (shown in green) and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin - Microtubule Marker (Alexa Fluor® 594), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

This image is courtesy of Carl Hobbs, King's College London, United Kingdom

Immunohistochemical detection (formaldehyde/paraffin-embedded sections) of vimentin protein using vimentin antibody - Neural Stem Cell Marker (ab45939) on human ovarian carcinoma sections.

Antigen retrieval step:Heat mediated. Blocking step: 1% BSA for 10 mins at RT°C. Ab45939 was used at 1/700 for 1 hour.

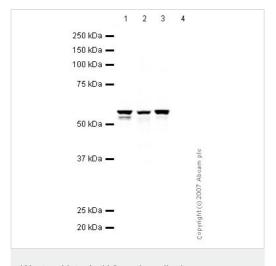
Secondary Antibody: Biotin-conjugated anti rabbit IG (1/300).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

Image courtesy of Mike Forbes by Abreview.

ab45939 staining Vimentin in murine kidney tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). Tissue was fixed with formaldehyde and a heat mediated antigen retrieval step was performed. Samples were then blocked with 10% serum for 30 minutes at room temperature followed by incubation with the primary antibody at a 1/2100 dilution for 18 hours at 4°C. A biotin-conjugated goat anti-rabbit polyclonal was used as secondary antibody at a 1/200 dilution.



Western blot - Anti-Vimentin antibody -Cytoskeleton Marker (ab45939) **All lanes :** Anti-Vimentin antibody - Cytoskeleton Marker (ab45939) at 1  $\mu$ g/ml

**Lane 1 :** HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lane 3 : HEK293 Human embryonic kidney cell line Whole Cell Lysate

**Lane 4 :** Ramos (Human Burkitt's lymphoma cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

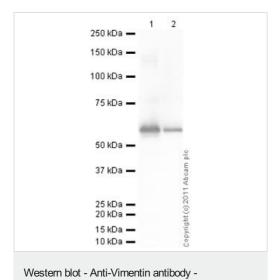
#### Secondary

**All lanes :** IRDye 680 Conjugated Goat Anti-Rabbit lgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 54 kDa

Observed band size: 54 kDa



Cytoskeleton Marker (ab45939)

**All lanes :** Anti-Vimentin antibody - Cytoskeleton Marker (ab45939) at 1  $\mu$ g/ml

**Lane 1 :** Recombinant Human Vimentin protein ( $\underline{ab73843}$ ) at 0.1  $\mu g$ 

**Lane 2 :** Recombinant Human Vimentin protein ( $\underline{ab73843}$ ) at 0.01  $\mu g$ 

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

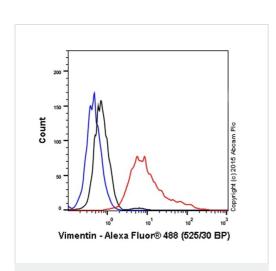
**Predicted band size:** 54 kDa **Observed band size:** 54 kDa

Exposure time: 10 seconds

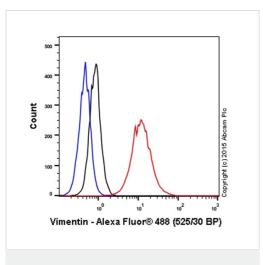
Ab45939 recognizes full length recombinant Human vimentin (ab73843) which has an expected molecular weight of 54 kDa.

Overlay histogram showing MDA-MB-231 cells stained with ab45939 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab45939, 0.01 $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit lgG (H&L) (ab150081) at 1/4000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (polyclonal) (ab171870, 0.01 $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

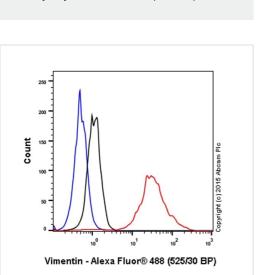
Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry (Intracellular) - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)



Flow Cytometry (Intracellular) - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)



Flow Cytometry (Intracellular) - Anti-Vimentin antibody - Cytoskeleton Marker (ab45939)

Overlay histogram showing NIH3T3 cells stained with ab45939 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab45939, 0.1 $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit lgG (H&L) (ab150081) at 1/4000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (polyclonal) (ab171870, 0.1 $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Overlay histogram showing SV40LT-SMC cells stained with ab45939 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab45939, 0.01 $\mu$ g/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit lgG (H&L) (ab150081) at 1/4000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (polyclonal) (ab171870, 0.01 $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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