abcam

Product datasheet

Anti-Vimentin antibody [V9] - Cytoskeleton Marker ab8069

敲除验证

28 Abreviews 152 References 14 图像

概述

产品名称	Anti-Vimentin抗体[V9] - Cytoskeleton Marker
描述	小鼠单克隆抗体[V9] to Vimentin - Cytoskeleton Marker
宿主	Mouse
经测试应用	**适用于:** ICC/IF, IHC-P, WB, Flow Cyt (Intra)
种属反应性	**与反应:** Rat, Human **预测可用于:** Horse, Chicken, Cow, Cat, Dog, Pig
免疫原	Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: A549, Hap1, HeLa, U-2 OS, human tonsil and MOLT4 whole cell lysates. ICC/IF: HeLa, Hap1, SKOV-3 and rat glial cells. IHC-P: Human kidney and oral cavity tissue sections. Flow Cyt (Intra): HAP1, MDA-MB-231 and SV40LT-SMC cells.
常规说明	This monoclonal antibody to vimentin has been knockout validated in ICC/IF. The expected staining was observed in wild type cells and no staining was seen in vimentin knockout cells. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine

纯度	Protein G purified
克隆	单克隆
克隆编号	V9
同种型	IgG1

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab8069于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF	★★★★★ (5)	Use a concentration of 0.5 - 5 µg/ml.
IHC-P	★★★★★ (9)	Use a concentration of 0.5 - 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	★★★★★ (5)	Use a concentration of 1 µg/ml. Detects a band of approximately 57 kDa (predicted molecular weight: 54 kDa).
Flow Cyt (Intra)		Use 0.1-1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

靶标

功能 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.

组织特异性 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.

疾病相关 Cataract 30

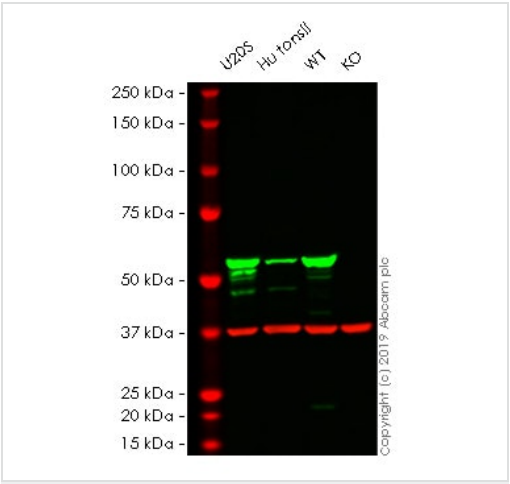
序列相似性 Belongs to the intermediate filament family.

结构域 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.

翻译后修饰 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.
细胞定位	Cytoplasm.
形式	Vimentin is found in connective tissue and in the cytoskeleton.

图片



Western blot - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

All lanes : Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069) at 1 µg/ml

Lane 1 : U-2 OS (Human bone osteosarcoma epithelial cell line) cell lysate
Lane 2 : Human tonsil cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : VIM knockout HeLa cell lysate

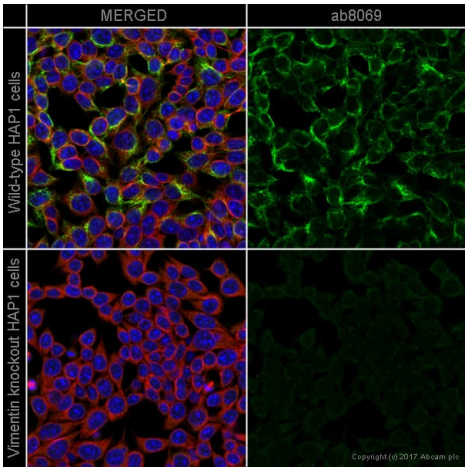
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 54 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab8069 observed at 53 kDa. Red - loading control, **ab181602** observed at 37 kDa.

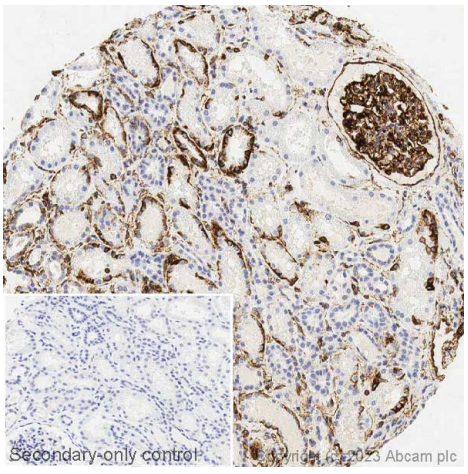
ab8069 was shown to react with Vimentin in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab255446** (knockout cell lysate **ab263775**) was used. Wild-type and Vimentin knockout samples were subjected to SDS-PAGE. ab8069 and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 µg/ml and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

ab8069 staining Vimentin (colored green) in wild-type HAP1 cells (top panel) and VIM 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 ab8069 at 0.5µg/ml and **ab202272** (Rabbit monoclonal [EP1332Y] to alpha Tubulin (Alexa Fluor® 594)) at 1/250 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with **ab150117** (Goat secondary antibody to Mouse IgG (Alexa Fluor® 488)) at 2 µg/ml (colored green). Nuclear DNA was labelled in blue with DAPI.

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



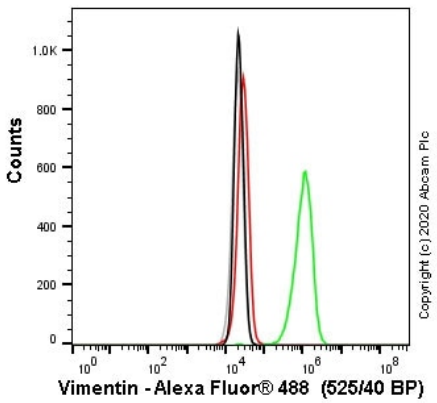
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

Lab

IHC image of Vimentin staining in a section of formalin-fixed paraffin-embedded human normal kidney* performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab8069, 0.5ug/ml, 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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.



Flow Cytometry (Intracellular) - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

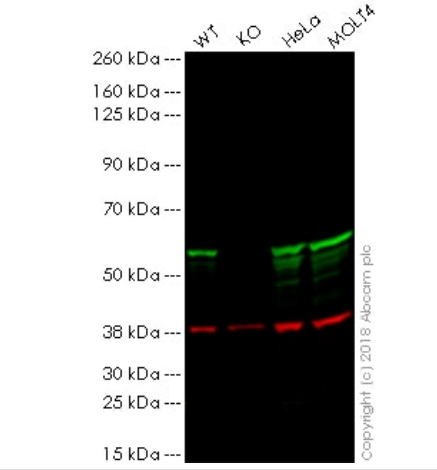
Flow cytometry overlay histogram showing wild-type HAP1 (green line) and VIM knockout HAP1 cells stained with ab8069 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab8069) ($1x10^6$ in 100 µl at 0.04 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-mouse IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150117**) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody was mouse IgG1&kappa (**ab170190**) used at the same concentration and conditions as the primary antibody (wild-type HAP1 - black line VIM knockout HAP1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min) / permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.



Western blot - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

All lanes : Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069) at 1 µg/ml

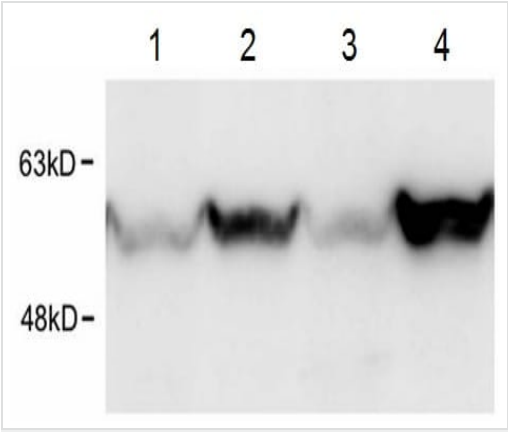
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : VIM (Vimentin) knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : MOLT4 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 54 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab8069 observed at 57 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab8069 was shown to specifically react with Vimentin in wild-type HAP1 cells as signal was lost in VIM (Vimentin) knockout cells. Wild-type and VIM (Vimentin) knockout samples were subjected to SDS-PAGE. Ab8069 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/10000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed **ab216772** and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed **ab216777** secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



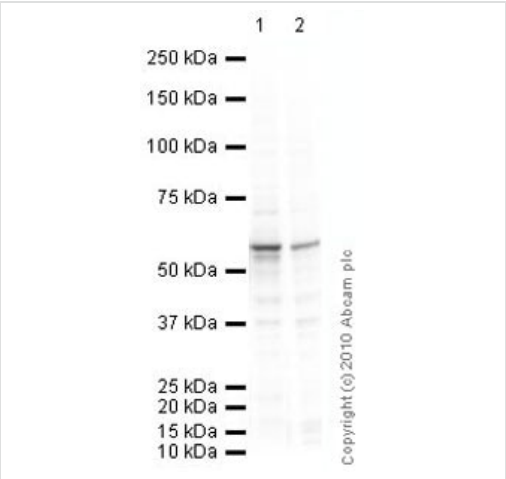
Western blot - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

Image from Tange S et al., PLoS One. 2014;9(12):e115684. Fig 9(H).; doi: 10.1371/journal.pone.0115684. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

All lanes : Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

Lane 1 : Whole cell lysate of A549 cells
Lane 2 : Whole cell lysate of A549 cells treated with TGF-beta
Lane 3 : Whole cell lysate of A549 cells overexpressing JARID2
Lane 4 : Whole cell lysate of A549 cells overexpressing JARID2 treated with TGF-beta

Predicted band size: 54 kDa



Western blot - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

All lanes : Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069) at 1 µg/ml

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) Whole Cell Lysate
Lane 2 : MOLT4 (Human lymphoblastic leukemia cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

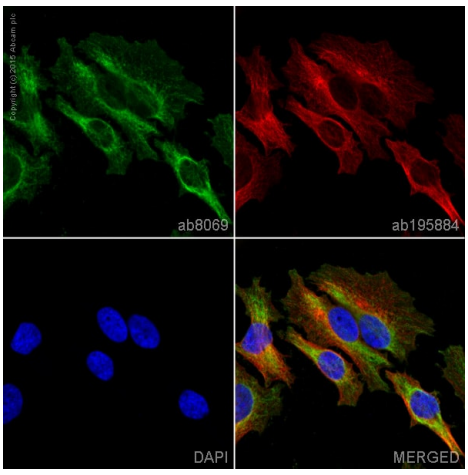
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

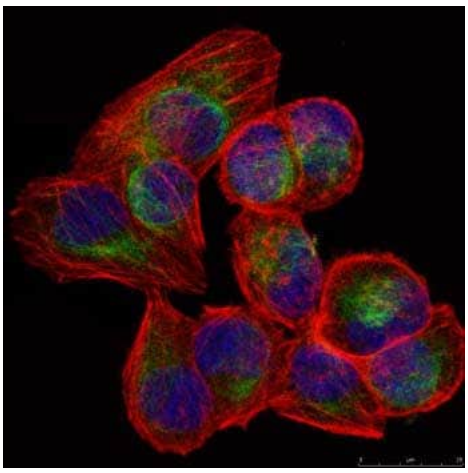
Predicted band size: 54 kDa
Observed band size: 57 kDa

Exposure time: 20 minutes



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

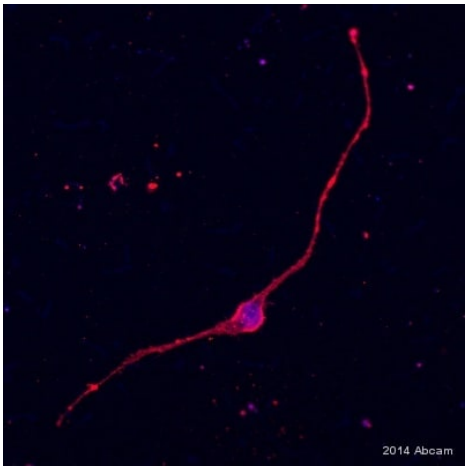
ab8069 staining Vimentin in HeLa cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab8069 at 1/100 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 2µg/ml (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 [V9] - Cytoskeleton Marker (ab8069)

Image from Loessner D et al, Biomaterials. 2010 Nov;31(32):8494-506. Epub 2010 Aug 14. doi:10.1016/j.biomaterials.2010.07.064

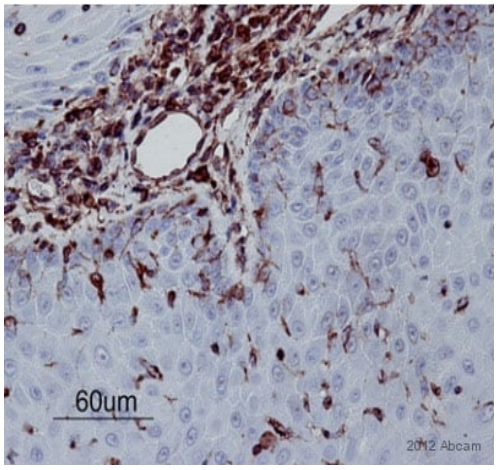
ab8069 staining Vimentin in human epithelial ovarian serous adenocarcinoma cell line SKOV-3 by Immunocytochemistry/ Immunofluorescence.
Samples were fixed with 4% PFA in PBS pH 7.4 and then permeabilised using 0.2% saponin for 30 minutes. A blocking step was performed using 1% BSA/PBS for 1 hour. Samples were then incubated with ab8069 at a 1/200 dilution in 1% BSA/PBS for 1 hour. The secondary antibody was a goat anti-mouse Alexa 488 (green) diluted 1/1000, 1% BSA/PBS for 1 hour. Samples were then incubated with phalloidin (red for actin staining) in 1% BSA/PBS for 45 minutes and counterstained with DAPI (blue for nuclei staining) in PBS for 45 minutes.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

This image is courtesy of an anonymous Abreview

ab8069 staining Vimentin in rat glial cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 25°C. Samples were incubated with primary antibody (1/200) for 10 hours at 4°C. An Alexa Fluor® 555-conjugated anti-mouse IgG polyclonal (1/300) was used as the secondary antibody.

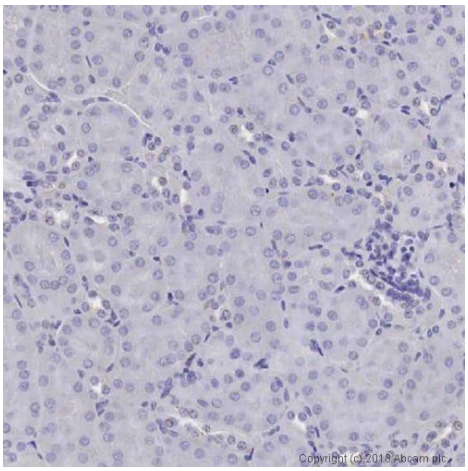


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

This image is courtesy of an anonymous Abreview

ab8069 staining Vimentin in Human oral cavity tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

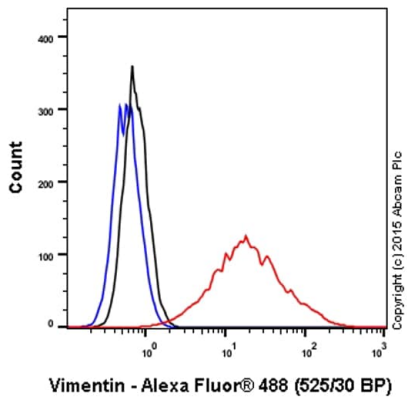
Tissue was fixed with paraformaldehyde and blocked with **ab64226** Protein Block for 5 minutes at 25°C; antigen retrieval was by heat mediation in pH 6 buffer . Samples were incubated with primary antibody (1/1000 in 10% NGS) for 16 hours at 4°C. An undiluted biotinylated goat anti-rabbit polyclonal IgG was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

IHC image showing no staining when ab8069 was used on **mouse kidney** formalin fixed paraffin embedded tissue, using MOM detection kit, **ab127055**. The section was pre-treated using pressure cooker heat mediated antigen retrieval with sodium citrate buffer (pH6) for 30 mins. The section was incubated with ab8069 at 5 µg/ml for 15 mins at room temperature. DAB was used as the chromogen. The section was counterstained with haematoxylin and mounted with DPX.

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.

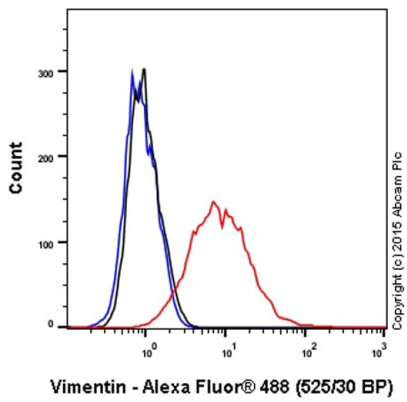


Flow Cytometry (Intracellular) - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

Overlay histogram showing SV40LT-SMC cells stained with ab8069 (red line). The cells were fixed with 4% formaldehyde (10 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 (ab8069, 0.1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (**ab150113**) at 1/2000 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 0.1µg/1x10^6 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.

This antibody gave a positive signal in SV40LT-SMC cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Triton X-100 for 20 min used under the same conditions.



Flow Cytometry (Intracellular) - Anti-Vimentin antibody [V9] - Cytoskeleton Marker (ab8069)

Overlay histogram showing MDA-MB-231 cells stained with ab8069 (red line). The cells were fixed with 4% formaldehyde (10 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 (ab8069, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (**ab150113**) at 1/2000 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 1µg/1x10^6 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.

This antibody gave a positive signal in MDA-MB-231 cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Triton X-100 for 20 min used under the same conditions.

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