abcam

Product datasheet

# Anti-Vimentin antibody [SP20] ab16700

KO VALIDATED Recombinant RabMAb

7 Abreviews 88 References 9 Images

## Overview

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|---|---|
| **Product name** | Anti-Vimentin antibody [SP20] |
| **Description** | Rabbit monoclonal [SP20] to Vimentin |
| **Host species** | Rabbit |
| **Specificity** | We have data to show that ab16700 is not suitable for work on mouse tissue. For researchers working on mouse we recommend using **ab92547**. If you would like further information on this, please do not hesitate to contact our technical support team. |
| **Tested applications** | **Suitable for:** ICC/IF, WB, IHC-P, Flow Cyt |
| **Species reactivity** | **Reacts with:** Human<br>**Predicted to work with:** Rat, Hamster, Cow, Xenopus laevis |
| **Immunogen** | Recombinant full length protein corresponding to Human Vimentin aa 1 to the C-terminus. |
| **Positive control** | WB: U-2 OS, Hu tonsil and HeLa cell lysates. Flow Cyt: HeLa cells. ICC/IF: HAP1-VIM cells, human limbal epithelial cells. IHC-P: Human breast cancer and melanoma tissue. IHC-Fr: Colorectal cancer tissue. |
| **General notes** | This product has switched from a hybridoma to recombinant production method on 4th September 2023.<br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br>**This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.** |

## Properties

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|---|---|
| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. |

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| **Storage buffer** | pH: 7.20<br>Preservative: 0.1% Sodium azide<br>Constituents: 98.9% PBS, 1% BSA |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | SP20 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab16700 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 |
|---|---|---|
| **ICC/IF** | ★★★★★ (3) | 1/1000. |
| **WB** | ★★★★★ (1) | Use at an assay dependent concentration. Predicted molecular weight: 53 kDa. |
| **IHC-P** | | 1/200. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. |
| **Flow Cyt** | | 1/100. |

## Target

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| **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.<br>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.<br>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.<br>Phosphorylated at Ser-56 by CDK5 during neutrophil secretion in the cytoplasm. Phosphorylated by STK33.<br>O-glycosylated during cytokinesis at sites identical or close to phosphorylation sites, this interferes with the phosphorylation status. |

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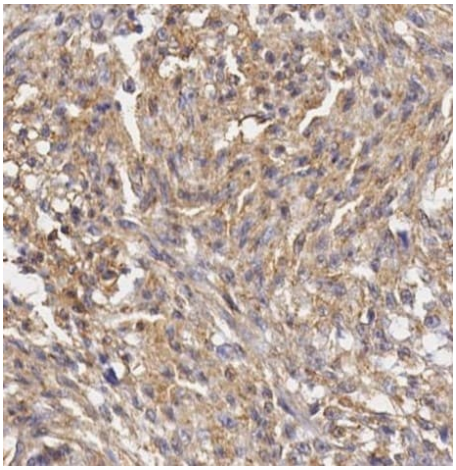
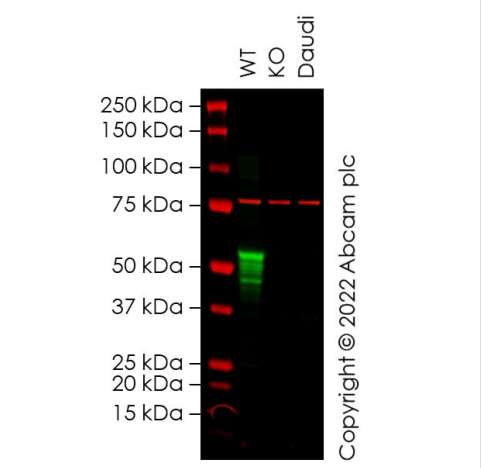
| | S-nitrosylation is induced by interferon-gamma and oxidatively-modified low-densitity lipoprotein (LDL(ox)) possibly implicating the iNOS-S100A8/9 transnitrosylase complex. |
| **Cellular localization** | Cytoplasm. |
| **Form** | Vimentin is found in connective tissue and in the cytoskeleton. |

## Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vimentin antibody [SP20] (ab16700)

Immunohistochemical analysis of paraffin-embedded Human melanoma tissue labeling Vimentin with ab16700 at 1/200 dilution. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.




Western blot - Anti-Vimentin antibody [SP20] (ab16700)

**All lanes :** Anti-Vimentin antibody [SP20] (ab16700) at 1/120 dilution

**Lane 1 :** Wild-type A549 cell lysate
**Lane 2 :** Vimentin knockout A549 cell lysate
**Lane 3 :** Daudi cell lysate

Lysates/proteins at 20 µg per lane.

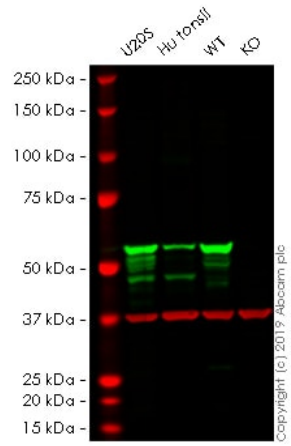
Performed under reducing conditions.

**Predicted band size:** 53 kDa
**Observed band size:** 55 kDa

**This image was generated using a previous batch manufactured using the hybridoma production method.**

False colour image of Western blot: Anti-Vimentin antibody [SP20] staining at 1/120 dilution, shown in green; Mouse anti-CANX

[CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab16700 was shown to bind specifically to Vimentin. A band was observed at 55 kDa in wild-type A549 cell lysates with no signal observed at this size in VIM knockout cell line **ab288984**. To generate this image, wild-type and VIM knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween$®$ 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.




Western blot - Anti-Vimentin antibody [SP20] (ab16700)

**All lanes :** Anti-Vimentin antibody [SP20] (ab16700) at 1/100 dilution

**Lane 1 :** U20S 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.

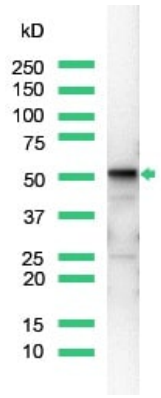
**Predicted band size:** 53 kDa

**This image was generated using a previous batch manufactured using the hybridoma production method.**

**Lanes 1 - 4:** Merged signal (red and green). Green - ab16700 observed at 53 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab16700 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. ab16700 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 100 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room

temperature before imaging.

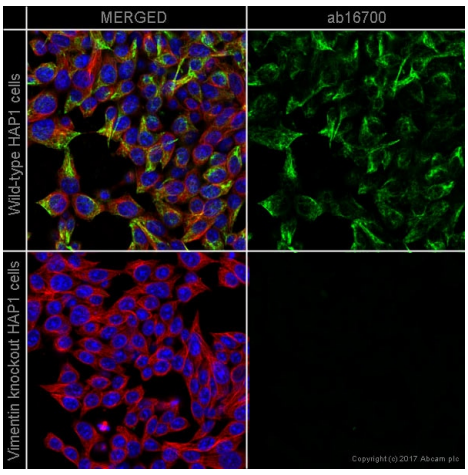



Western blot - Anti-Vimentin antibody [SP20] (ab16700)

Anti-Vimentin antibody [SP20] (ab16700) at 1/100 dilution + HeLa cell lysate

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

**This image was generated using a previous batch manufactured using the hybridoma production method.**

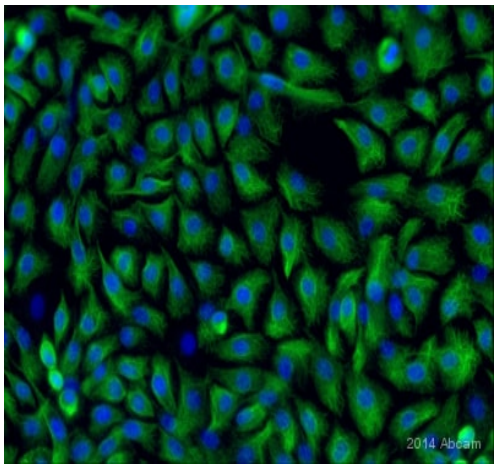



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [SP20] (ab16700)

**This image was generated using a previous batch manufactured using the hybridoma production method.**

ab16700 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 ab16700 at 1/1000 dilution and **ab195889** at 1/250 dilution (shown in pseudo-color red) overnight at +4°C. The cells were then incubated with **ab150081** (Goat polyclonal Secondary Antibody to 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).

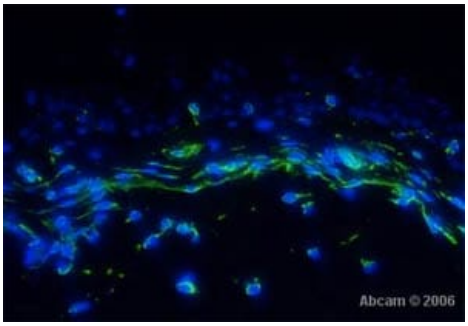



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [SP20] (ab16700)

This image is courtesy of an anonymous Abreview

**This image was generated using a previous batch manufactured using the hybridoma production method.**

ab16700 staining Vimentin in human corneal limbal epithelial cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.3% Triton X-100 for 5 minutes. Samples were incubated with primary antibody (1/200 in PBS + 10% normal goat serum) for 18 hours at 4°C. An Alexa Fluor®488-conjugated goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

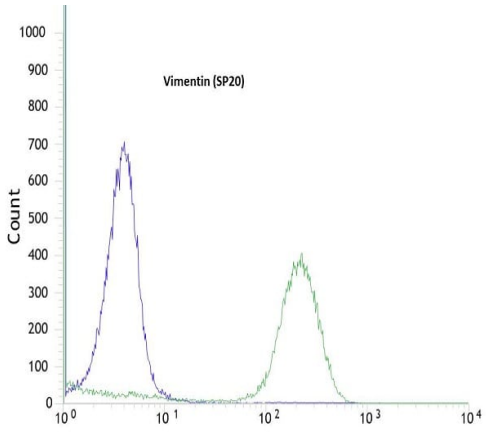



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [SP20] (ab16700)

This image is courtesy of an Abreview submitted by Miss Szu-Yu Chen

**This image was generated using a previous batch manufactured using the hybridoma production method.**

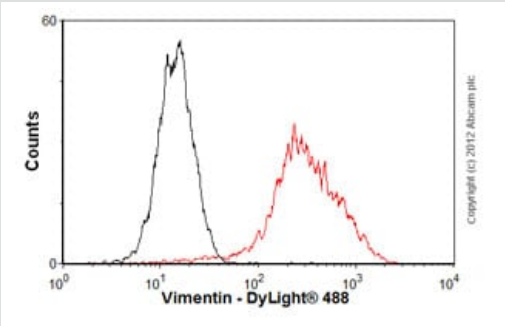
**ab16700** at 1/200 staining Human Limbal Epithelial Cells by ICC/IF. The cells were incubated with the antibody for 1 hour and then a FITC conjugated goat antibody was used as the secondary. The image shows vimentin staining in green and hoechst staining in blue. The upper cells in the image (vimentin negative) are epithelium cells. the vimentin positive cells are stroma cells.




Flow Cytometry - Anti-Vimentin antibody [SP20] (ab16700)

**This image was generated using a previous batch manufactured using the hybridoma production method.**

Flow cytometric analysis of rabbit anti-Vimentin (SP20) antibody ab16700 (1/100) in HeLa cells (green) compared to negative control of rabbit IgG (blue).




Flow Cytometry - Anti-Vimentin antibody [SP20] (ab16700)

**This image was generated using a previous batch manufactured using the hybridoma production method.**

Overlay histogram showing HeLa cells stained with **ab16700** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween 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 (ab16700, 1/100 dilution) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (**ab96899**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.



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