

Product datasheet

Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker ab92547

KO VALIDATED Recombinant RobMAb

***** 91 Abreviews 1492 References 32 Images

Overview	
Product name	Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker
Description	Rabbit monoclonal [EPR3776] to Vimentin - Cytoskeleton Marker
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P, mIHC
Species reactivity	Reacts with: Mouse, Rat, Human, African green monkey
	Predicted to work with: Cat, Pig, Rhesus monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK293, Jurkat, A549, NIH3T3, HUVEC and COS-1 cell lysates; mouse and rat brain tissue lysates. IHC-P: Human kidney, colon, breast adenocarcinoma, cervical carcinoma and ovarian cancer tissues, mouse brain and kidney, E17 rat cheek and rat skin tissue sections; Rhesus monkey retina tissue. IHC-Fr: Mouse testis tissue. ICC/IF: HeLa, human adenocarcinoma, human schlemms canal endothelium and wild-type HAP1 cells. Flow Cyt (intra): HeLa cells. mIHC: Human testis
General notes	 This product is a recombinant monoclonal antibody, which offers several advantages including: High batch-to-batch consistency and reproducibility Improved sensitivity and specificity Long-term security of supply Animal-free production For more information see here. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents. We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

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.
Dissociation constant (K _D)	$K_{D} = 1.10 \times 10^{-10} M$
	LOW 10 ⁻⁶ -7 -8 -9 -10 -11 -12 Learn more about K _D
o:	
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Storage buffer Purity	Preservative: 0.01% Sodium azide
	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA Protein A purified

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab92547 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
Flow Cyt (Intra)		1/50 - 1/500. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★ ★ ★ ★ ★ (20)	Use a concentration of 2 μ g/ml. This product gave a positive signal in HeLa (VIM knockout HeLa cells were used as a negative control) fixed with 4% formaldehyde (10 min) and 100% methanol (5 min).
WB	★ ★ ★ ★ ★ ★ ★ (<u>14)</u>	1/1000 - 1/5000. Predicted molecular weight: 54 kDa.
IHC-P	★ ★ ★ ★ ★ <u>(37)</u>	1/200 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
mIHC		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.
Tissue specificity	Involved with LARP6 in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2. Highly expressed in fibroblasts, some expression in T- and B-lymphocytes, and little or no

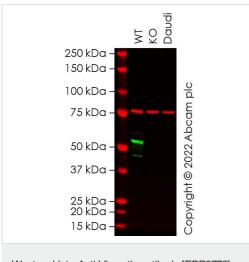
	expression in Burkitt's lymphoma cell lines. Expressed in many hormone-independent mammary carcinoma cell lines.
Involvement in disease	Cataract 30
Sequence similarities	Belongs to the intermediate filament family.
Domain	The central alpha-helical coiled-coil rod region mediates elementary homodimerization. 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-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

Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	× (stromal cells 🖌)	Human placenta	×	Clear cell carcinoma of human kidney	×	Human glioma	×.
Human cerebrum	* (endothelial cells 🗸)	Human skeletal muscle	× (stromal cells ✓)	Human bladder cancer	× (stromal cells ✓)	Human hepatocellular carcinoma	× (stromal cells 🖌)
Human colon	× (stromal cells ✓)	Human skin	× (stromal cells ✓)	Human breast carcinoma	*	Human lung carcinoma	1
ıman endometrium	×	Human spleen	×	Human cervical carcinoma	*	Human ovarian carcinoma	<i>.</i>
Human kidney	<u> </u>	Human stomach	× (stromal cells ✓)	Human colon carcinoma	× (stromal cells ✓)	Human pancreatic carcinoma	×.
Human liver	× (stromal cells ✓)	Human testis	×	Human endometrial carcinoma	×	Human prostatic hyperplasia	× (stromal cells ✓)
Human lung	1	Human thyroid	×	Human gastric adenocarcinoma	× (stromal cells ✓)	Human thyroid carcinoma	1
luman mammary gland	<u> </u>	Human tonsil	×				
Human pancreas	1						

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) Tissue Microarrays stained for Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker using ab92547 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested. The section was incubated with ab92547 at 4°C overnight followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer) secondary antibody (**ab214880**).

Heat mediated antigen retrieval was performed using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0)



Western blot - Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker (ab92547)

All lanes : Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) at 1/1000 dilution

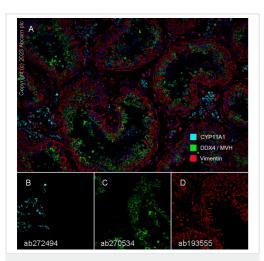
Lane 1 : Wild-type A549 cell lysate Lane 2 : VIM knockout A549 cell lysate Lane 3 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 54 kDa Observed band size: 55 kDa

False colour image of Western blot: Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (ab238078) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab92547 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.



Multiplex immunohistochemistry - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

This data was developed using <u>ab193555</u>, the same antibody clone in a different buffer formulation.

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) analysis of human testis tissue.

Panel A: Merged staining of anti-Vimentin (**ab193555**; red; Opal[™]690), anti-CYP11A1 (**ab272494**; cyan; Opal[™]520) and anti-DDX4 / MVH (**ab270534**; green; Opal[™]570) on human testis.

Panel B: Anti-CYP11A1 stained on Leydig cells.

Panel C: Anti-DDX4 / MVH stained on all spermatogenic cell types.

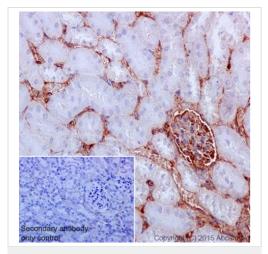
Panel D: Anti-Vimentin stained on Sertoli cells and fibroblasts.

Key protocol steps: The section was incubated in three rounds of staining: in the order of <u>ab193555</u> (1:2000 dilution) and <u>ab272494</u> (1:10000 dilution) for 30 mins, then <u>ab270534</u> (1:2000 dilution) for 10 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal[™] 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

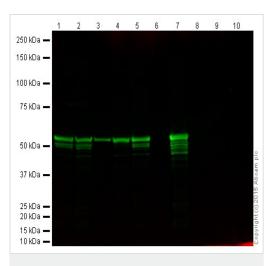
DAPI was used as a nuclear counter stain. Opal Polymer HRP Ms + Rb was used as a secondary.

Antigen retrieval: Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemical staining of paraffin embedded mouse kidney with purified ab92547 at a working dilution of 1/250. The secondary antibody used is <u>Goat Anti-Rabbit IgG H&L (HRP) (ab97051)</u> <u>secondary antibody</u> at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)



Western blot - Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker (ab92547) **All lanes :** Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) at 1/1000 dilution (unpurified)

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

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

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

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

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

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

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

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

Lane 9 : Daudi (Human Burkitt's lymphoma cell line) Whole cell lysate

Lane 10 : Caco 2 (Human colorectal adenocarcinoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

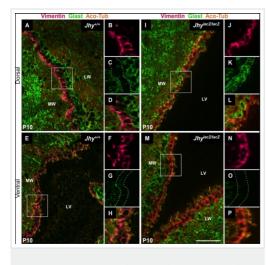
Performed under reducing conditions.

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 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.

Jhy^{JacZ/lacZ} mice exhibit delayed radial glial to ependymal cell differentiation.

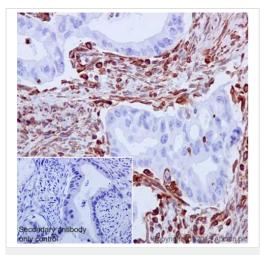
Immunohistochemical analysis of P10 lateral ventricle coronal sections from $Jhy^{+/+}$ (A, E) and $Jhy^{JacZ/IacZ}$ (I. M) mice for expression of Vimentin (pink, ab92547), Glast (green) and Acα-Tub (orange) in dorsal (A-D, I-L) and ventral (E-H, M-P) brain regions. Lower right panels (D, L, H, P) represent a higher magnification view of the merged image. In $Jhy^{+/+}$, medial wall dorsal and ventral cells express the differentiated ependymal markers Vimentin (A, B, E, F) and Acα-Tub (A, D, E, H), but are negative for the radial glial marker Glast (A, C, E, G). In JhylacZ/lacZ brains, some dorsal cells remain positive for the undifferentiated marker Glast (I, K), while also expressing the differentiated markers Vimentin and Aca-Tub (I, J, L). Jhy^{lacZ/lacZ} ventral cells express only Vimentin and Aca-Tub (M-P). The dotted line indicates the medial wall ependymal cells in (C, G, K, O). (Q-R) Graphical representation of the percentage of Glast(-)Vimentin(+)Acα-Tub(+) (black bar) and Glast(+)Vimentin(+)Aca-Tub(+) (grey bar) cells in dorsal (Q) and ventral (R) ependymal cells. MW, medial wall; LW, lateral wall; LV, lateral ventricle; * denotes p≤0.05. Scale bars: 50µm (A-P).



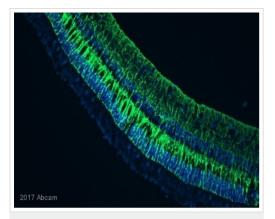
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody

[EPR3776] - Cytoskeleton Marker (ab92547)

Image from Muniz-Talavera H and Schmidt JV., PLoS One. 2017;12(12):e0184957. Fig 3.; doi: 10.1371/journal.pone.0184957. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

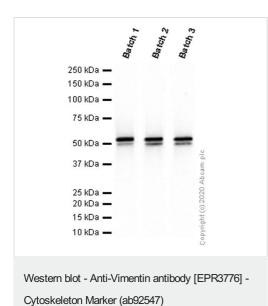


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified ab92547 at a working dilution of 1/250. The secondary antibody used is HRP goat anti-rabbit IgG H&L (<u>ab97051</u>) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) This image is courtesy of an anonymous Abreview Immunohistochemical staining of paraffin

embedded paraformaldehyde fixed rhesus monkey retina tissue with ab92547 (green) at a working dilution of 1/200. The sample was incubaded with the primary antibody fro 20 hours, at 4°C in 2.5% serum. The secondary antibody used is a Goat anti-rabbit AlexaFluor 488 at 1/400. Heat mediated antigen retrieval was perfomed using citrate pH 6. Tissue was blocked with 5% serum for 1 hour 30 minutes at 25°C



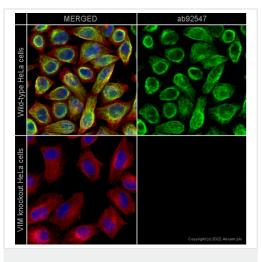
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

Different batches of ab92547 were tested on HEK-293 (Human embryonic kidney epithelial cell) lysate at 0.02 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 54 kDa.

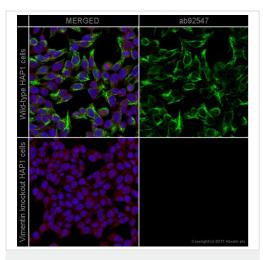
IHC image of unpurified ab92547 staining Vimentin in human breast adenocarcinoma formalin-fixed paraffin-embedded tissue sections*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab92547, 1/200 dilution, 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 negative 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



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

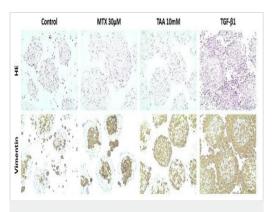


Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

ab92547 staining VIM in wild-type HeLa cells, with negative expression in VIM knockout HeLa cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised 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 ab92547 at 2 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin at 0.5 µg/ml. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor[®] 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150119**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor[®] 647), preadsorbed at 1/1000 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown..This product also work with 100% methanol (5 min) fixation under the same testing conditions.

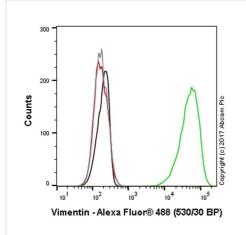
ab92547 staining Vimentin in wild-type HAP1 cells (top panel) and VIM knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab92547 at 0.5µg/ml and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody** at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

Image from Prestigiacomo V et al., PLoS One. 2017;12(6):e0179995. Fig 7.; doi: 10.1371/journal.pone.0179995.

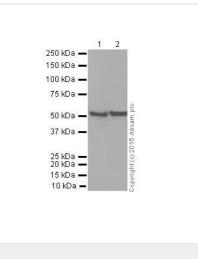


Flow Cytometry (Intracellular) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) Immunostaining of formalin fixed paraffin embedded human microtissues after exposure to MTX, TAA and TGF- β 1.

Formalin fixed paraffin embedded slides of HepaRG/THP-1 macrophages/hTERT-HSC microtissues were stained with Hematoxylin & Eosin (H&E) and vimentin after 14 days of treatment with MTX, TAA and TGF- β 1. Microtissues were fixed in 4% PFA and embedded in 2% agarose prior to paraffinization. Microtissues showed increase in the vimentin positive cells after MTX, TAA and TGF- β 1 exposure. Vimentin stainings show proliferation of stellate cells and THP-1 macrophages in the microtissues, suggesting the onset of inflammation process.

For full image see PMID 28665955.

Overlay histogram showing HAP1 wildtype (green line) and HAP1-VIM knockout cells (red line) stained with ab92547. 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 / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab92547, 0.5µg/ml) for 30 min at 22°C. The secondary antibody used wasGoat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) preadsorbed (**ab150081**) secondary antibodyat 1/2000 dilution for 30 min at 22°C. A Rabbit IgG isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-VIM knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.



Western blot - Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker (ab92547)

All lanes : Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) at 1/5000 dilution (purified)

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate Lane 2: HEK293 (Human epithelial cell line from embryonic kidney) cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

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

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST

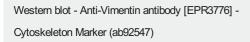
All lanes : Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) at 1/5000 dilution (purified)

Lane 1 : Mouse brain lysate Lane 2 : Rat brain lysate

Secondary

All lanes : HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

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



2

250 kDa -150 kDa 🗕 100 kDa 🗕

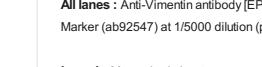
75 kDa 🗕

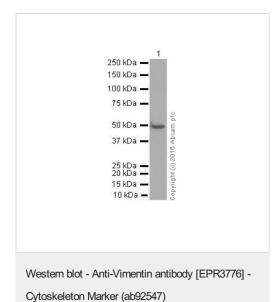
50 kDa 🗕 37 kDa 🗕

25 kDa — 20 kDa —

15 kDa 🗕 10 kDa 🗕

> Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST





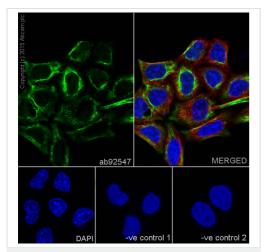
Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) at 1/20000 dilution (purified) + COS-1 (African green monkey kidney fibroblast-like cell line) cell lysate at 20 µg

Secondary

HRP goat anti-rabbit IgG (H+L) at 1/1000 dilution

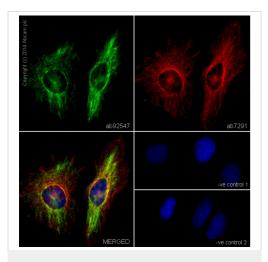
Predicted band size: 54 kDa Observed band size: 54 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST

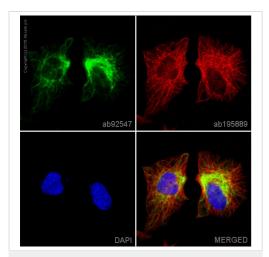


Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

Immunofluorescence staining of HeLa (human epithelial cell line from cervix adenocarcinoma) cells with purified ab92547 at a working dilution of 1/250, counter-stained with DAPI. The secondary antibody was Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody, used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) 1/1000, shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified ab92547 was used at a dilution of 1/500 followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) at a dilution of 1/500. For negative control 2, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at a dilution of 1/400.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

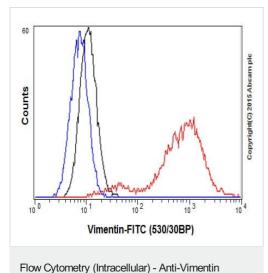


Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) ab92547 staining Vimentin in HeLa (human epithelial cell line from cervix adenocarcinoma) cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab92547 at 5µg/ml and <u>ab7291</u> at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with <u>Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)</u> <u>preadsorbed (ab150081) secondary antibody</u> at 2 µg/ml (shown in green) and <u>Goat Anti-Mouse IgG H&L (Alexa Fluor® 594)</u> <u>preadsorbed (ab150120)</u> at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Negative controls: 1– Rabbit primary antibody and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.

Unpurified ab92547 staining Vimentin in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab92547 at a working concentration of 5µg/ml and **ab195889**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at 1/250 overnight at +4°C, followed by a further incubation at room temperature for 1h with **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081)** secondary antibody at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

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



antibody [EPR3776] - Cytoskeleton Marker (ab92547)

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) Overlay histogram showing HeLa cells fixed in 2% PFA and stained with purified ab92547 at a dilution of 1 in 50 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).

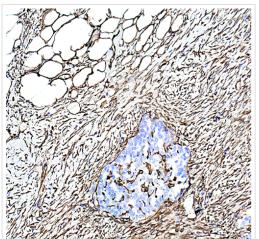
Anti-vimentin (ab92547) staining in E17 rat cheek sections using immunohistochemistry (formaldehyde-fixed, paraffin-embedded sections). Heat-mediated antigen retrieval was carried out using citric acid. Samples were incubated with primary antibody (1/2000) for two hours at room temperature. A biotin-conjugated goat antirabbit IgG polyclonal was used as the secondary antibody. Image courtesy of Mr Carl Hobbs, Kings College London.



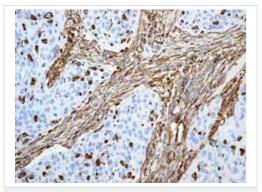
Immunohistochemistry (Formalin/PFA-fixed paraffin-

embedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) Anti-vimentin (ab92547) staining in adult mouse brain (the dentate gyrus region of the hippocampus) using immunohistochemistry (formaldehyde-fixed, paraffin-embedded sections). Heat-mediated antigen retrieval was carried out using citric acid. Samples were incubated with primary antibody (1/2000) for two hours at room temperature. A biotin-conjugated goat anti-rabbit IgG polyclonal was used as the secondary antibody.

Image courtesy of Mr Carl Hobbs, Kings College London.

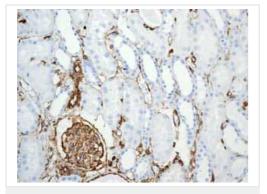


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) Anti-vimentin (ab92547) staining in human ovarian cancer tissue using immunohistochemistry (formaldehyde-fixed, paraffinembedded sections). Heat-mediated antigen retrieval was carried out using citric acid. Samples were incubated with primary antibody (1/2000) for two hours at room temperature. A biotin-conjugated goat anti-rabbit IgG polyclonal was used as the secondary antibody. Image courtesy of Mr Carl Hobbs, Kings College London.



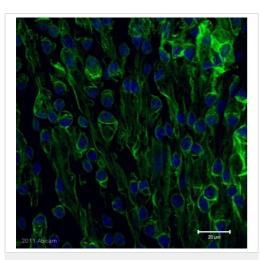
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

Immunohistochemical analysis of formalin/PFA-fixed paraffinembedded human cervical carcinoma tissue sections labeling Vimentin with ab92547.



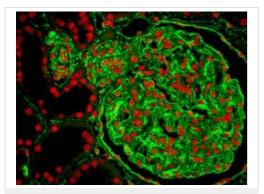
Immunohistochemical analysis of formalin/PFA-fixed paraffinembedded human kidney tissue sections labeling Vimentin with ab92547.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

This image is courtesy of an Abreview submitted by Thomas Read

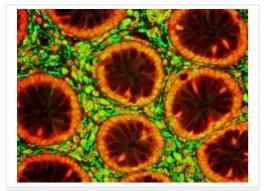


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)

Unpurified ab92547 staining vimentin in human Schlemms Canal Endothelium cells by ICC/IF

(Immunocytochemistry/immunofluorescence). Cells were fixed with formaldehyde, permeabilized with Triton X-100 0.2% and blocked with 10% serum for 30 minutes at 20°C. Samples were incubated with primary antibody (1/200 in DPBS) for 3 hours at 20°C. An undiluted Alexa Fluor[®]488-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.

Fluorescent immunohistochemical analysis of paraffin-embedded human normal kidney tissue using unpurified ab92547. Green-Vimentin red-Pl.

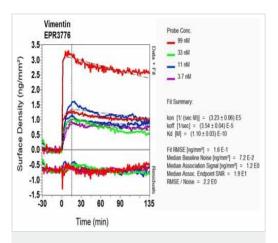


Fluorescent immunohistochemical analysis of paraffin-embedded human normal colon tissue using unpurified ab92547. Green-Vimentin red-PI

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab92547) This image is courtesy of an anonymous Abreview.



OI-RD Scanning - Anti-Vimentin antibody [EPR3776]

- Cytoskeleton Marker (ab92547)

ab92547 staining Vimentin in rat skin tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with 10% buffered normal formalin and blocked with 5% serum for 60 minutes at 21°C; antigen retrieval was by heat mediation in a 10mM Sodium citrate buffer. Samples were incubated with primary antibody (1/200 in blocking buffer) for 12 hours at 4°C. A Cy3[®]-conjugated donkey anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody.

Equilibrium dissociation constant (K_D) Learn more about K_D

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Marker (ab92547)

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