### abcam

#### Product datasheet

# Anti-Vimentin antibody [RV202] - Cytoskeleton Marker ab8978



\*\*\*\* 44 Abreviews 507 References 21 Images

#### Overview

Product name Anti-Vimentin antibody [RV202] - Cytoskeleton Marker

**Description** Mouse monoclonal [RV202] to Vimentin - Cytoskeleton Marker

Host species Mouse

**Specificity** This antibody reacts exclusively with vimentin, which is expressed in mesenchymal cells and

mesenchymal derived tumors e.g. lymphoma, sarcoma and melanoma.

Tested applications Suitable for: IHC-Fr, WB, IHC-P, Flow Cyt (Intra), ICC/IF

**Species reactivity** Reacts with: Mouse, Rat, Human, Pig, Zebrafish

Immunogen Recombinant full length protein corresponding to Bovine Vimentin. RV202 is a mouse monoclonal

lgG1 antibody derived by fusion of SP2/0-Ag14 mouse myeloma cells with spleen cells from a

BALB/c mouse immunized with a vimentin extract of bovine lens.

Database link: P08670

Positive control IHC-Fr: Human lung, Ed18 rat, pig colon and zebrafish embryo. ICC/IF: Human colon, rat brain,

SK-N-SH, human pancreatic adenocarcinoma cells and HAP1 cells. WB: NIH-3T3 and normal human dermal fibroblasts cell extracts. IHC-P: Human placenta, human small intestine, human spleen, human tonsillar lymphoma, human colon tissue, human fetal kidney tissue, dog soft tissue

sarcoma tissue. Flow Cyt (intra): HeLa cells.

General notes Vimentin (57 kDa) is the intermediate filament protein (IFP) of mesenchymal cells. This IFP

however often deviates from the tissue-specific and developmentally regulated pattern of expression. Besides its typical expression in most cultured cells, vimentin is also expressed together with several other IFPs during early stages of development. As differentiation proceeds, vimentin is exchanged for the tissue-specific intermediate filament type. Also in cancers, vimentin

is often expressed in addition to the tissue-specific IFP.

RV202 is a mouse monoclonal lgG1 antibody derived by fusion of SP2/0-Ag14 mouse myeloma cells with spleen cells from a BALB/c mouse immunized with a cytoskeletal vimentin extract of calf

lens.

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

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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

#### **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** Preservative: 0.09% Sodium azide

Constituent: PBS

Purity Protein G purified

**Clonality** Monoclonal

Clone number RV202

Myeloma Sp2/0-Ag14

**Isotype** lgG1

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab8978 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
IHC-Fr	<b>★★★★★ (4)</b>	Use at an assay dependent concentration.  Recommended range is 1/100 - 1/200 for Immunohistochemistry with avidin-biotinylated horseradish peroxidase complex (ABC) as detection. For PFA fixed tissue use at 1/1000.
WB	<b>★★★★★ (4)</b>	Use at an assay dependent concentration. Predicted molecular weight: 53 kDa.
IHC-P	<b>★★★★★</b> (13)	1/100 - 1/1000.
Flow Cyt (Intra)		1/100 - 1/200.
ICC/IF	**** (19)	Use a concentration of 1 µg/ml.

#### **Target**

**Function** 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.

#### **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

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.

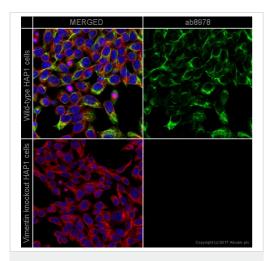
Cellular localization

Cytoplasm.

**Form** 

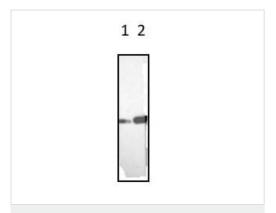
Vimentin is found in connective tissue and in the cytoskeleton.

#### **Images**



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978) ab8978 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 ab8978 at 1µg/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 lgG (Alexa Fluor® 488)) 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).



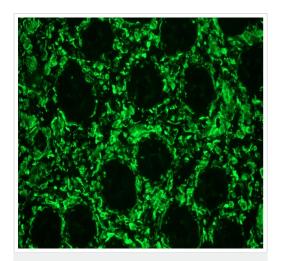
Western blot - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

**All lanes :** Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Lane 1: NIH3T3 cell lysate

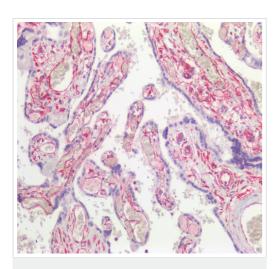
Lane 2: Normal human dermal fibroblasts cell extracts.

**Predicted band size:** 53 kDa **Observed band size:** 57 kDa



Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

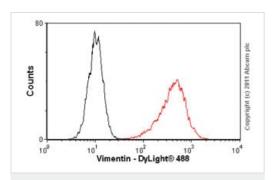
Immunohistochemistry analysis of frozen section of pig colon labeling Vimentin with ab8978, showing positive staining in connective tissue cells and no reactivity in epithelial cells.



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

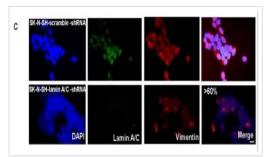
[RV202] - Cytoskeleton Marker (ab8978)

Immunohistochemistry analysis of formalin fixed, paraffin embedded section of human placenta labeling Vimentin with ab8978, showing positive staining in connective tissue cells and no reactivity in epithelial cells.



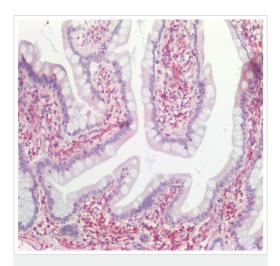
Flow Cytometry (Intracellular) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Overlay histogram showing HeLa cells stained with <u>ab8978</u> (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Triton 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 (ab8978, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (<u>ab96879</u>) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (<u>ab91353</u>, 2µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed. This anti-Vimentin antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Triton used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Rauschert, I. et al PLoS One. 2017 Apr 19;12(4):e0175953. doi: 10.1371/journal.pone.0175953. eCollection 2017 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/



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

[RV202] - Cytoskeleton Marker (ab8978)

## Silencing of Lamin A/C in unmethylated neuroblastoma cells induces changes in different cytoskeletal components

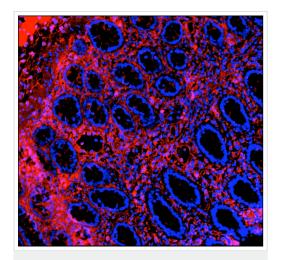
Immunofluorescence staining showing changes in SK-N-SH-lamin-A/C-shRNA compared with SK-N-SH-scramble-shRNA in (A)  $\beta$ -actin filaments, (B) F-actin filaments. (C) Vimentin filaments, and (D)  $\alpha$ -tubulin. Lamin A/C is shown in green;  $\beta$ -actin, F-actin, vimentin, and  $\alpha$ -tubulin are shown in red.DNA is stained in blue (DAPI). Scale bar,  $10\mu$ M.

Vimentin is detected using ab8978 at 1/100 dilution.

Neuroblastoma cells were fixed with 4% paraformaldehyde and permeabilized using 0.5% Triton X-100.

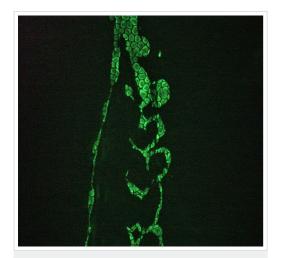
(From Figure 5C of Rauschert et al)

Immunohistochemistry analysis of formalin fixed, paraffin embedded section of human small intestine labeling Vimentin with ab8978; showing positive staining in connective tissue cells and no reactivity in epithelial cells.



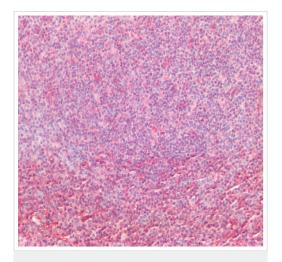
Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Immunohistochemistry analysis of frozen section of pig colon labeling Vimentin with ab8978, showing positive staining in connective tissue cells and no reactivity in epithelial cells. Nuclear staining with DAPI.



Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

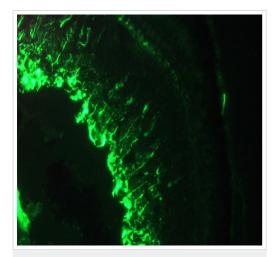
Immunohistochemistry analysis of frozen section of zebrafish embryo labeling Vimentin with ab8978.



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

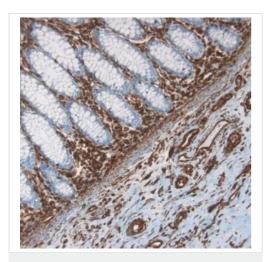
[RV202] - Cytoskeleton Marker (ab8978)

Immunohistochemistry analysis of formalin fixed, paraffin embedded section of human spleen labeling Vimentin with ab8978, showing positive staining in connective tissue cells and lymphoid cells.



Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

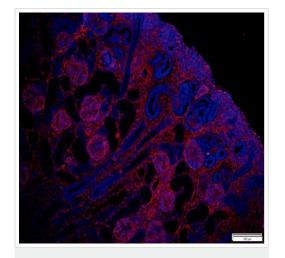
Immunohistochemistry analysis of frozen section of zebrafish embryo labeling Vimentin with ab8978.



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

[RV202] - Cytoskeleton Marker (ab8978)

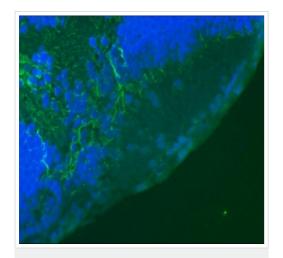
Paraffin-embedded human colon tissue stained for Vimentin using ab8978 at 1/100 dilution in immunohistochemical analysis.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody
[RV202] - Cytoskeleton Marker (ab8978)

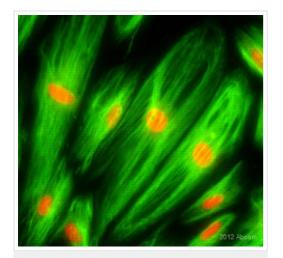
This image is courtesy of an anonymous abreview.

ab8978 staining Vimentin in human fetal kidney tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with formaldehyde and blocked with CAS-Block for 1 hour at 25°C; antigen retrieval was by heat mediation using OmniPrep (pH 9). Samples were incubated with primary antibody (1/500) for 1 hour at 25°C. An Alexa Fluor® 555-conjugated Donkey polyclonal (1/200) was used as the secondary antibody.



Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

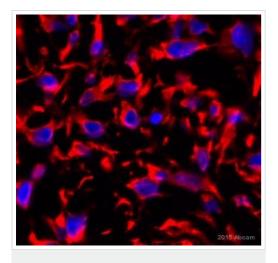
Immunofluorescence staining images of 9 day old zebrafish embryos. ab8978 reacts with in connective tissue cells and bloodvessels. Frozen sample treated with Acetone:Methanol 1:1, antibody diluted 1/100 and incubated for 45 minutes at room temperature.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

This image is courtesy of an Abreview submitted by Dr J. Chai

ab8978 staining Vimentin in human colon fibroblasts by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with methanol, permeabilized in 0.1% Triton and blocked with 0.25% serum free protein blocker for 20 minutes at 28°C. Samples were incubated with primary antibody (1/100 in antibody diluent) for 2 hours at 28°C. <a href="mailto:ab6785">ab6785</a> Goat polyclonal anti-Mouse IgG - H&L (FITC) (1/800) was used as the secondary antibody. Nuclei were counterstained with propidium iodide.

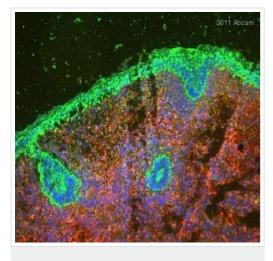


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Vimentin antibody

[RV202] - Cytoskeleton Marker (ab8978)

This image is courtesy of an anonymous abreview.

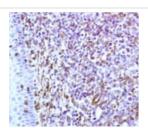
ab8978 staining Vimentin in dog soft tissue sarcoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehydefixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 15% serum for 1 hour at 20°C; antigen retrieval was by heat mediation in a Tris/EDTA pH9 buffer. Samples were incubated with primary antibody (1/100 in TBS) for 18 hours at 20°C. A Alexa Fluor® 647-conjugated Goat antimouse IgG polyclonal (1/400) was used as the secondary antibody.



Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

Image courtesy of an AbReview submitted by Dr Ruma Raha-Chowdhury

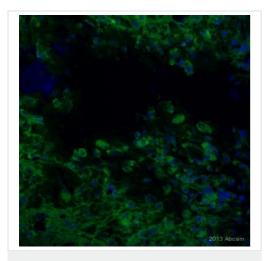
IHC-Fr image of Ed18 rat stained with ab8978. Fresh frozen sections were incubated in 10% normal donkey serum in 0.1% PBS- and 0.3% triton X100 for 1h to permeabilise the tissues and block non-specific protein-protein interactions. The sectons were then incubated with the ab8978 (1µg/ml) and ferroportin overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 donkey anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 568 (red) donkey anti-mouse at a 1/1000 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. Vimentin expressed in the gut muscles.



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

[RV202] - Cytoskeleton Marker (ab8978)

Immunohistochemistry analysis of formalin fixed, paraffin embedded section of human tonsillar lymphoma labeling Vimentin with ab8978.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

This image is courtesy of Dr. Ruma Raha-Chowdhury, Cambridge University, United Kingdom.

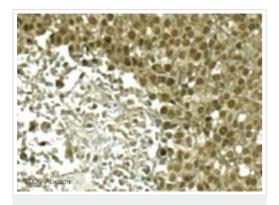
IHC-FoFr image of vimentin staining on rat injured cortical sections using ab8978 (1:500). The brain was perfusion fixed using 4% PFA and the sections were permeabilized using 0.1% TritonX in 0.1% PBS. The sections were then blocked using 10% donkey serum for 1 hour at 24°C. ab8978 was diluted 1:500 and incubated with sections for 24 hours using 4°C. The secondary antibody used was donkey polyclonal to rabbit IgG conjugated to Alexa Fluor 488.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

This image is courtesy of an Abreview submitted by Jennifer Dembinski

ab8978 staining vimentin in human pancreatic adenocarcinoma cells by immunocytochemistry/ immunofluorescence. Cells were PFA fixed and permeabilized in 0.2% Triton X prior to blocking in 3% BSA for 30 minutes at 24°C. The primary antibody was diluted 1/200 and incubated with the sample for 16 hours at 21°C. Alexa fluor® 488 mouse polyclonal to mouse lg, diluted 1/300 was used as the secondary antibody.



Immunohistochemistry (Frozen sections) - Anti-Vimentin antibody [RV202] - Cytoskeleton Marker (ab8978)

This image is courtesy of an anonymous abreview.

ab8978 staining Vimentin in human lung tissue section by Immunohistochemistry (Frozen sections). Tissue samples were fixed with formaldehyde and blocking with 5% commercially available blocking agent was performed at 37°C for 15 minutes. The sample was incubated with primary antibody (1/250) at 37°C for 1 hour. A HRP-conjugated Goat polyclonal to mouse IgG was used as secondary antibody at 1/1000 dilution.

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