abcam

Product datasheet

Anti-Vimentin antibody [VI-10] ab20346

KO VALIDATED

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Overview

Product name	Anti-Vimentin antibody [VI-10]
Description	Mouse monoclonal [VI-10] to Vimentin
Host species	Mouse
Specificity	The antibody VI-10 reacts with vimentin, a 57 kDa intermediate filament expressed in variety of mesenchymal and mesodermal cell types.
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Chicken, Human **Predicted to work with:** Pig
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: HAP1 cells; Mouse dissociated neural precursor cells; Chicken postnatal erythrocytes; RBL rat basophiilioc cells. IHC-P: Human skin tissue. WB: HAP1 cell lysate. Flow Cyt: NIH3T3 cells.
General notes	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

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	pH: 8.0 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Proprietary Purification

Purification notes	Purified by precipitation and chromatography.
Clonality	Monoclonal
Clone number	VI-10
Myeloma	unknown
Isotype	IgM
Light chain type	unknown

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab20346 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

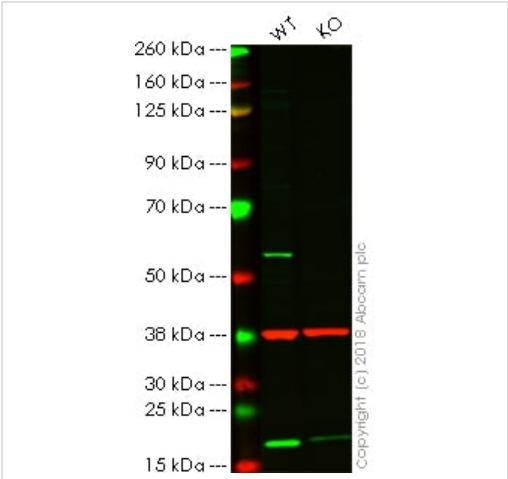
Application	Abreviews	Notes
WB	★★★★★ (4)	Use at an assay dependent concentration. Predicted molecular weight: 54 kDa.
IHC-P	★★★★★ (4)	Use a concentration of 1 - 5 µg/ml.
Flow Cyt	★★★★★ (1)	Use 1µg for 10^6 cells. **ab91545** - Mouse monoclonal IgM, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (7)	Use a concentration of 1 µg/ml. Staining technique: (a) fix cells for 10 min in methanol at -20°C and for 6 min in acetone at -20°C; (b) fix cells directly in methanol for 10 min at -20°C or in acetone for 10 min at -20°C.

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. 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 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



Western blot - Anti-Vimentin antibody [VI-10] (ab20346)

All lanes : Anti-Vimentin antibody [VI-10] (ab20346) at 1 µg/ml

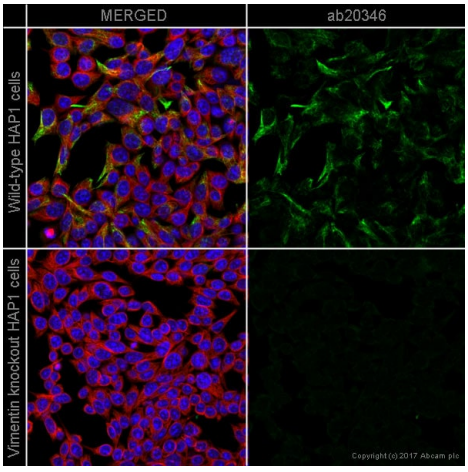
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : VIM (Vimentin) knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 54 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab20346 observed at 57 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

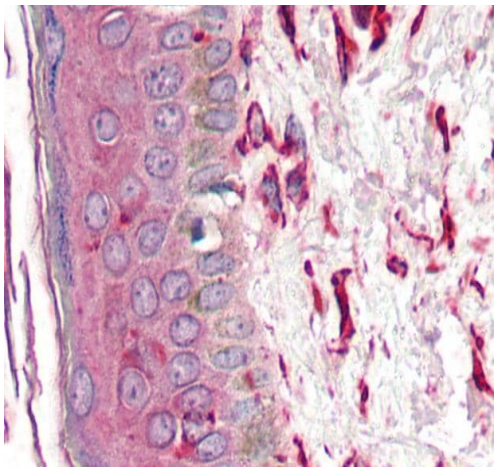
ab20346 was shown to specifically react with Vimentin in wild-type HAP1 cells as signal was lost in VIM (Vimentin) knockout cells. Wild-type and VIM (Vimentin) knockout samples were subjected to SDS-PAGE. Ab20346 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [VI-10] (ab20346)

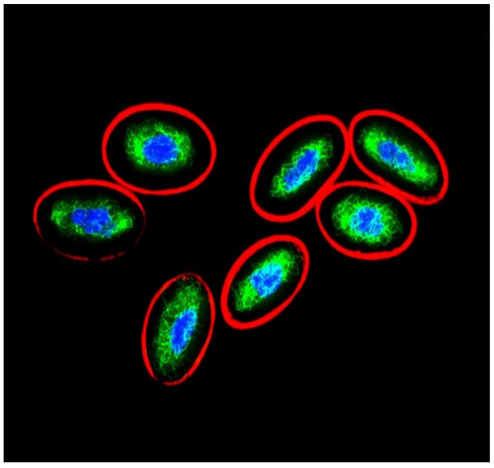
ab20346 staining Vimentin in wild-type HAP1 cells (top panel) and Vimentin knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab20346 at 1µg/ml dilution and **ab202272** at 1/250 dilution (shown in pseudo-color red) overnight at +4°C. The cells were then incubated with **ab150117** (Goat Anti-Mouse 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).



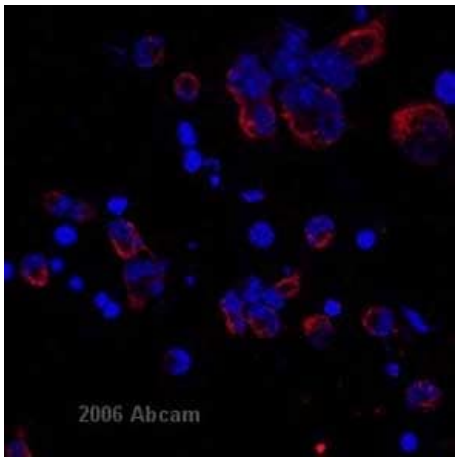
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vimentin antibody [VI-10] (ab20346)

Immunohistochemical analysis of formalin-fixed, paraffin-embedded human skin tissue labeling Vimentin with ab20346 at 5 ug/ml.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [VI-10] (ab20346)

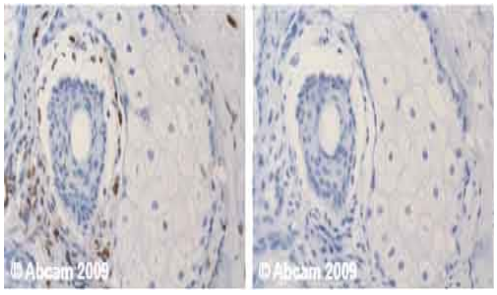
Immunocytochemical analysis of chicken postnatal erythrocytes labeling Vimentin with ab20346 at 1 ug/ml (green). Cells were counterstained with anti-alpha-tubulin (red) and DAPI (blue).



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [VI-10] (ab20346)

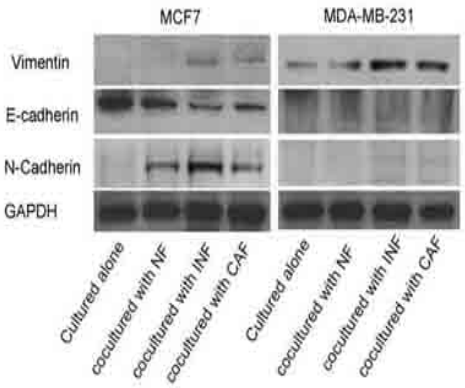
ab20346 at a 1/500 dilution staining mouse dissociated neural precursor cells by immunocytochemistry. The antibody was incubated with the cells for 1 hour and then detected using a goat **anti-mouse Alexa-Fluor 568** (red stain) **ab175473**>**ab175473**). The image also shows a blue nuclear counterstain. Confocal image is shown with 2x zoom detail in top right corner.

This image is courtesy of an Abreview submitted by **Randal Moldich** on **22 February 2006**.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Vimentin antibody [VI-10] (ab20346)

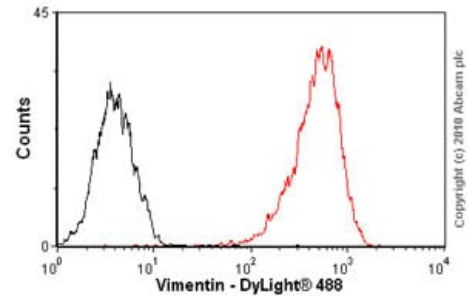
Human normal skin. Staining is localised to cytoplasm. Left panel: with primary antibody at 1 ug/mL. Right panel: isotype control. Sections were stained using an automated system DAKO Autostainer Plus , at room temperature: sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffers citrate pH6.1 in a DAKO PT Link. Slides were peroxidase blocked in 3% H_2O_2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for mouse for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Western blot - Anti-Vimentin antibody [VI-10] (ab20346)

Image from Gao MQ et al, J Cell Sci. 2010 Oct 15;123(Pt 20):3507-14. Epub 2010 Sep 14, Fig 3. DOI 10.1242/jcs.072900

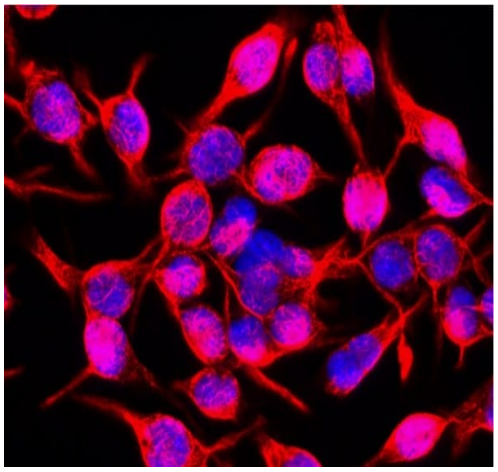
ab20346 used at a 1/1000 dilution in Western Blot.5-20µg of total protein from each sample was loaded.



Flow Cytometry - Anti-Vimentin antibody [VI-10] (ab20346)

Overlay histogram showing NIH3T3 cells stained with **ab20346** (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 (ab20346, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was a goat **anti-mouse DyLight® 488** (IgG; H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgM [ICIGM] (**ab91545**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in NIH3T3 cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Triton used under the same

conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Vimentin antibody [VI-10] (ab20346)

Immunofluorescence staining of RBL rat basophiilioc cell line with anti-Vimentin (VI--10). Nuclei are stained with DAPI (blue).

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