abcam

Product datasheet

Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker ab194719

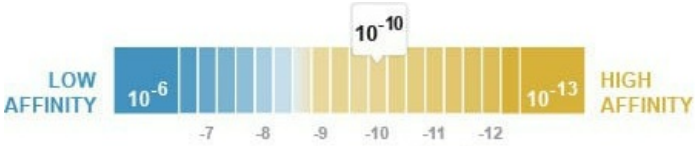
KO VALIDATED | Recombinant | RabMAb

5 References | 5 Images

Overview

Product name	Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker
Description	Alexa Fluor® 647 Rabbit monoclonal [EPR3776] to Vimentin - Cytoskeleton Marker
Host species	Rabbit
Conjugation	Alexa Fluor® 647. Ex: 652nm, Em: 668nm
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: NIH 3T3 and wildtype HAP1 cells. Flow Cyt (intra): NIH 3T3 cells and wildtype HAP1 cells.
General notes	Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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. Avoid freeze / thaw cycle. Store In the Dark.
Dissociation constant (K_D)	K_D = 1.10 x 10^{-10} M  Learn more about K_D
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR3776
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab194719 in the following tested applications.

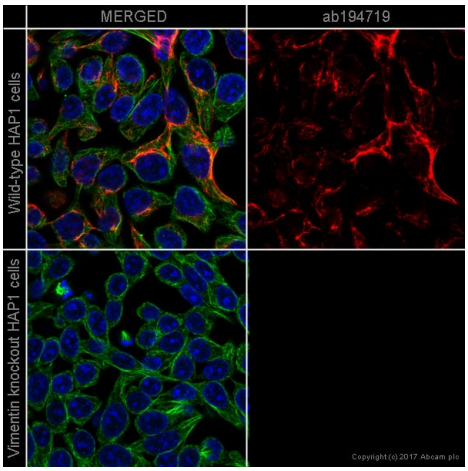
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500. **ab199093** - Rabbit monoclonal IgG (Alexa Fluor® 647), is suitable for use as an isotype control with this antibody.
ICC/IF		1/100 - 1/1000.

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.

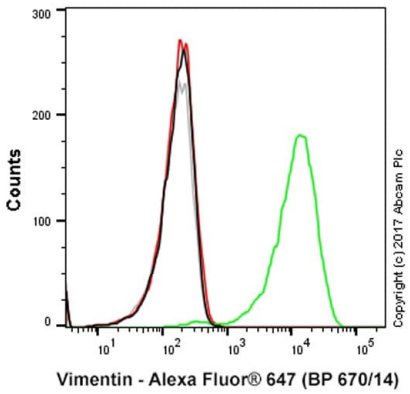
Images



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194719)

ab194719 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 ab194719 at 1/1000 dilution (shown in red) and **ab195887** at 1/250 dilution (shown in green) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

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



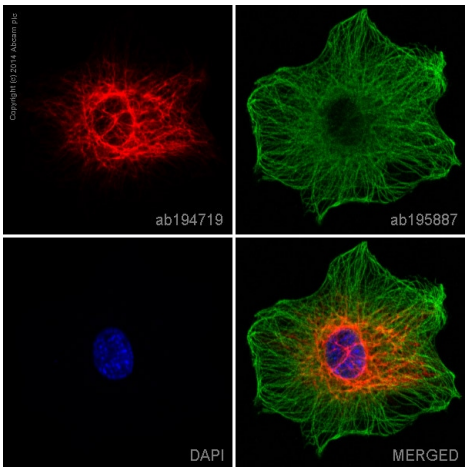
Flow Cytometry (Intracellular) - Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194719)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-VIM knockout cells (red line) stained with ab194719. 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 (ab194719, 0.01µg/ml dilution) for 30 min at 22°C.

A rabbit monoclonal IgG isotype control antibody (**ab199093**) 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 40 mW Yellow/Green laser (640nm) and 670/14 bandpass filter.

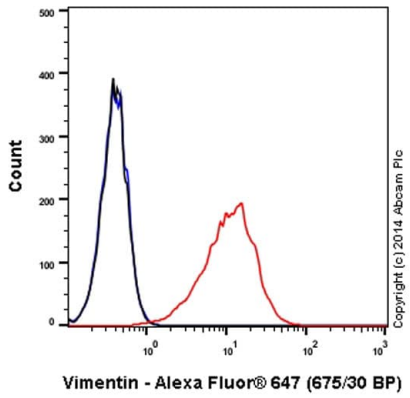
This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194719)

ab194719 staining Vimentin in NIH3T3 cells. The cells were fixed with 4% formaldehyde (10 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 ab194719 at 1/100 dilution(shown in red) and **ab195887**, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 488, shown in green) at 2µg/ml overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

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

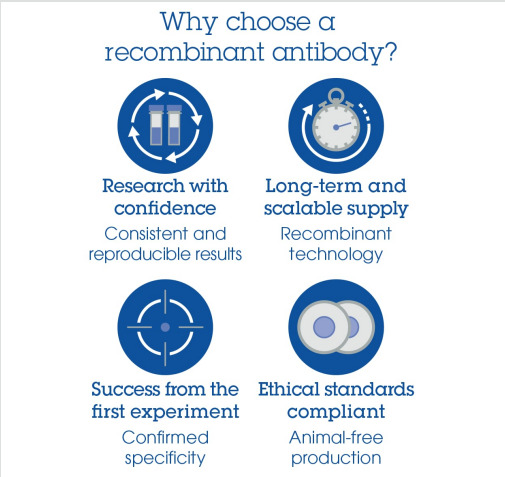


Flow Cytometry (Intracellular) - Alexa Fluor® 647 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194719)

Overlay histogram showing NIH3T3 cells stained with ab194719 (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 (ab194719, 1/500 dilution) for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 647 used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a solid-state 25mW red diode laser (635 nm) and 675/30 bandpass filter.

This antibody gave a positive signal in NIH3T3 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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