

Product datasheet

Alexa Fluor® 594 Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker ab154207

KO VALIDATED Recombinant RabMAb

14 References 5 Images

Overview		
Product name	Alexa Fluor® 594 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker	
Description	Alexa Fluor® 594 Rabbit monoclonal [EPR3776] to Vimentin - Cytoskeleton Marker	
Host species	Rabbit	
Conjugation	Alexa Fluor® 594. Ex: 590nm, Em: 617nm	
Tested applications	Suitable for: ICC/IF, Flow Cyt (Intra)	
Species reactivity	Reacts with: Mouse, Human	
	Predicted to work with: Rat	
Immunogen	Synthetic peptide corresponding to Human Vimentin aa 400 to the C-terminus (C terminal). Database link: <u>P08670</u>	
Positive control	ICC/IF: HeLa, NIH3T3 and wildtype HAP1 cells. Flow Cyt (intra): wildtype HAP1 cells.	
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 <u>see here</u> .	
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	Life Technologies Corporation, 5781 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@thermofisher.com.	

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.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3776
lsotype	lgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab154207 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		1/100 - 1/1000.
Flow Cyt (Intra)		Use a concentration of 0.1 µg/ml.

Target

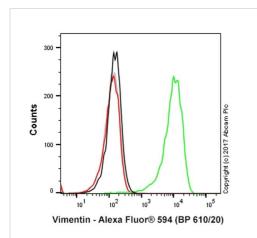
Function	Vimentins are class-III intermediate filaments found in various non-epithelial cells, especially mesenchymal cells. Vimentin is attached to the nucleus, endoplasmic reticulum, and mitochondria, either laterally or terminally. Involved with LARP6 in the stabilization of type I collagen mRNAs 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
	2

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



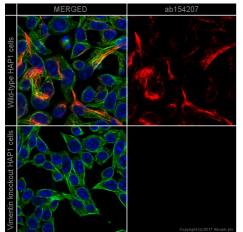
Flow Cytometry (Intracellular) - Alexa Fluor® 594 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab154207)

Overlay histogram showing HAP1 wildtype (green line) and HAP1-VIM knockout cells (red line) stained with ab154207. The cells were fixed with 4% formaldehyde (10 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 proteinprotein interactions followed by the antibody (ab154207, 0.1µg/ml dilution) for 30 min at 22°C.

A rabbit monoclonal IgG isotype control antibody (ab208568) 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 Yellow/Green laser (488nm) and 630/30 bandpass filter.

This antibody can also be used in HAP1 cells fixed with 80% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.

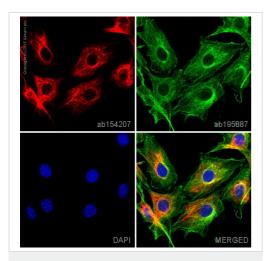


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 594 Anti-Vimentin antibody [EPR3776] -

Cytoskeleton Marker (ab154207)

ab154207 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 ab154207 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.

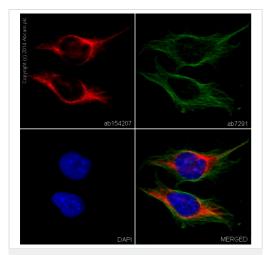
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



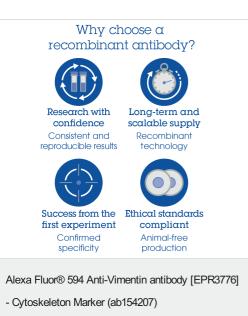
Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 594 Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker (ab154207) ab154207 staining Vimentin in NIH3T3 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab154207 at a 1/1000 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 488), at a 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

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

This product also gave a positive signal under the same testing conditions in NIH3T3 cells fixed with 100% methanol (5 min).



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 594 Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker (ab154207)



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ab154207 staining Vimentin in HeLa 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 ab154207 at a working dilution of 1 in 100 (shown in pseudo color red) and **ab7291** (Mouse monoclonal [DM1A] to alpha Tubulin) at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an AlexaFluor[®] 488 Goat anti-Mouse secondary (**ab150117**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

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