# abcam

## Product datasheet

# Alexa Fluor® 555 Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker ab203428





#### 3 References 4 Images

#### Overview

**Product name** Alexa Fluor® 555 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker

**Description** Alexa Fluor® 555 Rabbit monoclonal [EPR3776] to Vimentin - Cytoskeleton Marker

**Host species** Rabbit

Conjugation Alexa Fluor® 555, Ex: 555nm, Em: 565nm

**Tested applications** Suitable for: ICC/IF, Flow Cyt (Intra)

**Species reactivity** Reacts with: Mouse, Human

Predicted to work with: Rat

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Immunogen** 

Positive control ICC/IF: NIH3T3 and wildtype HAP1 cells. Flow Cyt (intra):wildtype HAP1 cells.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit General notes monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

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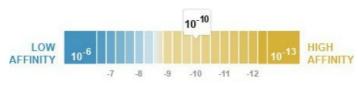
#### **Properties**

**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<sub>D</sub>)**  $K_D = 1.10 \times 10^{-10} M$ 



Learn more about K<sub>D</sub>

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 ab203428 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		1/1000. This product gave a positive signal in NIH3T3 cells fixed with 4% formaldehyde (10 min) and 100% methanol (5 min).
Flow Cyt (Intra)		Use a concentration of 0.1 - 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 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.

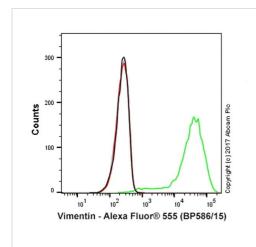
#### **Cellular localization**

Cytoplasm.

**Form** 

Vimentin is found in connective tissue and in the cytoskeleton.

#### **Images**

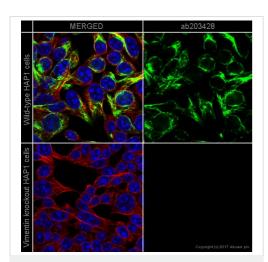


Flow Cytometry (Intracellular) - Alexa Fluor® 555 Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab203428) Overlay histogram showing HAP1 wildtype (green line) and HAP1-VIM knockout cells (red line) stained with ab203428. The cells were fixed with 80% methanol (5 min) and then permeabilized 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 (ab203428, 0.1µg/ml dilution) for 30 min at 22°C.

A rabbit monoclonal IgG isotype control antibody (<u>ab208569</u>) 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 (561nm) and 586/15 bandpass filter.

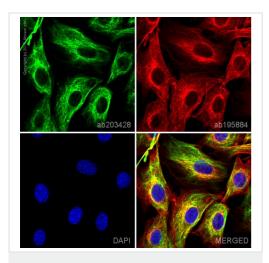
This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 555 Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker (ab203428)

ab203428 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 overnight at +4°C with ab203428 at a 1/1000 dilution (shown in green) and <a href="mailto:ab195884">ab195884</a>, Rat monoclonal [YOL1/34] to Tubulin (Alexa Fluor® 647), at a 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

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

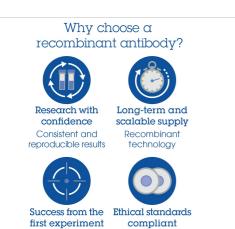


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 555 Anti-Vimentin antibody [EPR3776] -Cytoskeleton Marker (ab203428)

ab203428 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 ab203428 at a 1/1000 dilution (shown in green) and <u>ab195884</u>, Rat monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 647), at a 1/250 dilution (shown in red). 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).



Alexa Fluor® 555 Anti-Vimentin antibody [EPR3776]

Animal-free

production

- Cytoskeleton Marker (ab203428)

Confirmed

specificity

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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