abcam

Product datasheet

HRP Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker ab194718

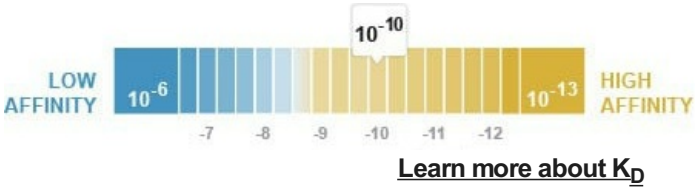
KO VALIDATED | Recombinant | RabMAb

4 Images

Overview

Product name	HRP Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker
Description	HRP Rabbit monoclonal [EPR3776] to Vimentin - Cytoskeleton Marker
Host species	Rabbit
Conjugation	HRP
Tested applications	**Suitable for:** IHC-P, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, A549 and HEK293 whole cell lysates. IHC-P: Human breast (fibroadenoma) tissue.
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)	$K_D = 1.10 \times 10^{-10}$ M  **Learn more about K_D**
Storage buffer	pH: 7.40 Preservative: 0.1% Proclin 300 Solution Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR3776
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab194718 in the following tested applications.

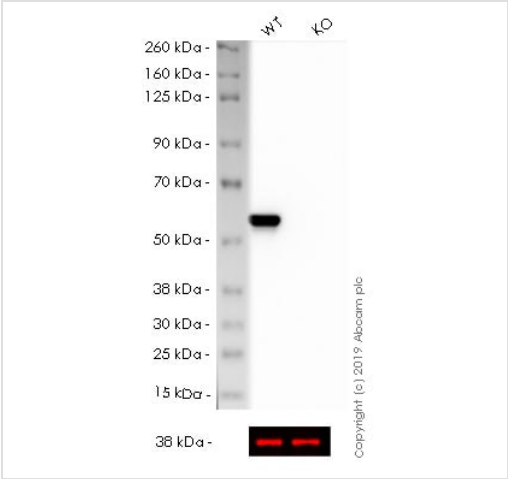
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. **ab199507** - Rabbit monoclonal IgG (HRP), is suitable for use an as isotype control with this antibody.
WB		1/1000. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).

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 - HRP Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194718)

All lanes : HRP Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194718) at 1/1000 dilution

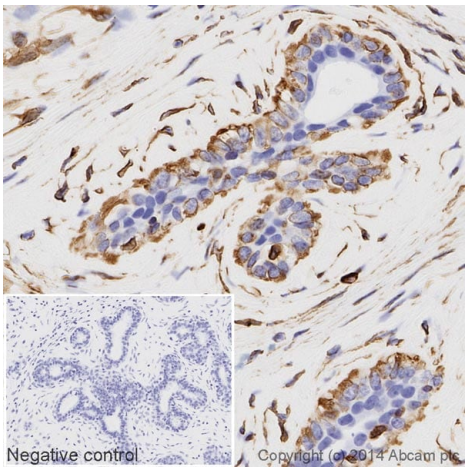
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : VIM knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 54 kDa

Exposure time: 150 seconds

ab194718 was shown to specifically react with VIM in wild-type HAP1 cells as signal was lost in VIM knockout cells. Wild-type and VIM knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab194718 and **ab184095** (Mouse monoclonal [mAbcam 9484] to GAPDH - Loading Control (Alexa Fluor® 680) loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. The loading control was imaged using the Licor Odyssey CLx prior to blots being developed with ECL technique.

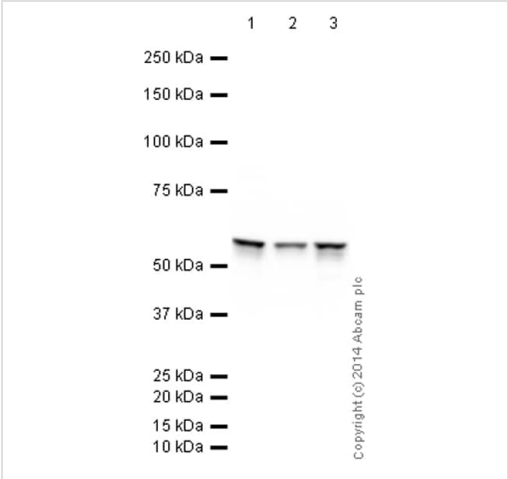


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - HRP Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194718)

IHC image of Vimentin staining in a section of formalin-fixed paraffin-embedded human breast (Fibroadenoma)*, performed on a Leica BOND. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab194718, 1/500 dilution, for 15 mins at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Western blot - HRP Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194718)

All lanes : HRP Anti-Vimentin antibody [EPR3776] - Cytoskeleton Marker (ab194718) at 1/1000 dilution

Lane 1 : HeLa whole cell lysate (**ab150035**)
Lane 2 : A549 (Human lung adenocarcinoma epithelial cell line) Whole Cell Lysate
Lane 3 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: 2 seconds

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab194718 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.

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