abcam

Product datasheet

Anti-Vimentin (phospho S73) antibody [EP1070Y] ab52944

RabMAb

4 References 6 Images

Overview

Product name Anti-Vimentin (phospho S73) antibody [EP1070Y]

Description Rabbit monoclonal [EP1070Y] to Vimentin (phospho S73)

Host species Rabbit

Tested applications Suitable for: WB, IP, Dot blot

Unsuitable for: ICC/IF or IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: HeLa; NIH/3T3; C6 lysates. IP: HeLa cells (untreated and Calyculin A treated).

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**.

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: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

Purity Protein A purified

Clonality Monoclonal

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Clone number EP1070Y

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab52944 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		1/10000. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).
IP		1/20. For unpurified use at 1:40.
Dot blot		Use at an assay dependent concentration.

Application notes Is unsuitable for ICC/IF or IHC-P.

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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 similaritiesBelongs 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

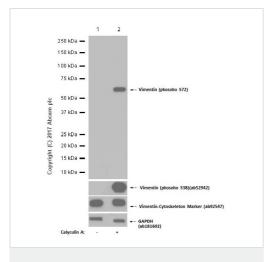
interferes with the phosphorylation status.

S-nitrosylation is induced by interferon-gamma and oxidatively-modified low-densitity lipoprotein

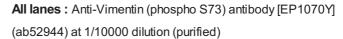
(LDL(ox)) possibly implicating the iNOS-S100A8/9 transnitrosylase complex.

Cellular localization Cytoplasm.

Form Vimentin is found in connective tissue and in the cytoskeleton.



Western blot - Anti-Vimentin (phospho S73) antibody [EP1070Y] (ab52944)



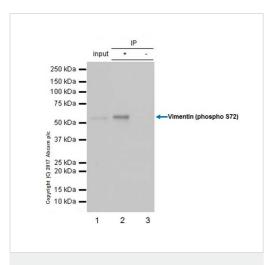
Lane 1 : C6 (Rat glial tumor cell line) whole cell lysate, untreated Lane 2 : C6 (Rat glial tumor glial cell) starved for 24 hours, then treated with 100ng Calyculin A for 60 minutes, whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 54 kDa



Immunoprecipitation - Anti-Vimentin (phospho S73) antibody [EP1070Y] (ab52944)

Blocking and diluting buffer: 5% NFDM/TBST

ab52944 (purified) at 1:20 dilution ($2\mu g$) immunoprecipitating Vimentin in HeLa starved 24 hours, then treated with 100nM Calyculin A for 30 minutes whole cell lysate.

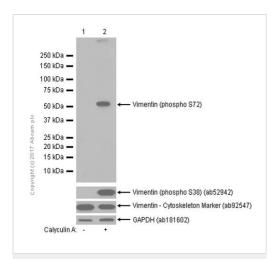
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) starved 24 hours,then treated with 100nM Calyculin A for 30 minutes whole cell lysate 10ug

Lane 2 (+): ab52944 & HeLa starved 24 hours, then treated with 100nM Calyculin A for 30 minutes whole cell lysate

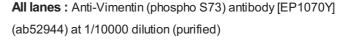
Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab52944 in HeLa starved 24 hours,then treated with 100nM Calyculin A for 30 minutes whole cell lysate

For western blotting, VeriBlot for IP Detection Reageant (HRP) (ab131366) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-Vimentin (phospho S73) antibody [EP1070Y] (ab52944)



Lane 1 : Untreated with HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 2: HeLa (Human cervix adenocarcinoma epithelial cell) starved for 24 hours,then treated with 100nM Calyculin A for 30 minutes whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 54 kDa

Blocking and diluting buffer: 5% NFDM/TBST

All lanes : Anti-Vimentin (phospho S73) antibody [EP1070Y] (ab52944) at 1/10000 dilution (purified)

Lane 1: Untreated with NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

Lane 2: NIH/3T3 (Mouse embryonic fibroblast) treated with 100ng/ml Calyculin A for 30 minutes whole cell lysates

Lysates/proteins at 15 µg per lane.

1 2 250 kDa — 150 kDa — 100 kDa — 75 kDa — 50 kDa — 25 kDa — 20 kDa — 115 kDa — 110 kDa — 15 kDa — 110 kDa — 15 kDa — 10 kDa — 15 kDa — 10 kDa — 110 kDa — Calyculin A: - + Vimentin (phospho S38) (ab52942) ← Vimentin - Cytoskeleton Marker (ab92547) ← GAPDH (ab181602)

Western blot - Anti-Vimentin (phospho S73) antibody [EP1070Y] (ab52944)

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 54 kDa

Blocking and diluting buffer: 5% NFDM/TBST



[EP1070Y] (ab52944)

Primary antibody dilution: 1/1000

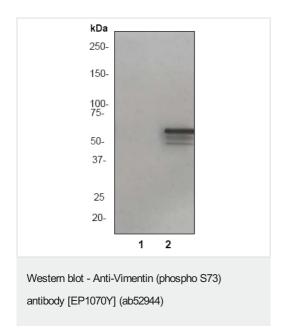
Secondary antibody: goat anti-rabbit lgG, (H+L), peroxidase

conjugated (1/2500 dilution)

Blocking & dilutiing buffer: 5% NFDM/TBST

Lane 1 sample: Vimentin (pS72) phospho peptide Lane 2 sample: Vimentin non-phospho peptide

Exposure time: 3 minutes



All lanes : Anti-Vimentin (phospho S73) antibody [EP1070Y] (ab52944) at 1/1000000 dilution

Lane 1: HeLa cell lysate untreated

Lane 2: HeLa cell lysate treated with Calyculin A

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: goat anti-rabbit HRP at 1/2000 dilution

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

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

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