Overview

Product name: Anti-Vimentin (phospho S55) antibody [4A4] ab22651
Description: Mouse monoclonal [4A4] to Vimentin (phospho S55)
Host species: Mouse
Specificity: Does not detect non-phosphorylated Vimentin or Vimentin phosphorylated by camp dependant kinase, protein kinase C or Ca²⁺ calmodulin dependant kinase II.
Tested applications: Suitable for: WB, ELISA, ICC/IF, IHC-P, IHC-Fr, Flow Cyt
Species reactivity: Reacts with: Mouse, Rat, Human, Ferret
Immunogen: Synthetic peptide: SLYSSSPGGAYV corresponding to amino acids 50 - 61 of Mouse Vimentin, containing phosphorlyated serine 55, and conjugated to KLH.
Positive control: The antibody was tested positive with the following cell lines via WB: U251, NIH-3T3, and 3Y1-B.
General notes: Abcam is committed to meeting high quality standards of ethical manufacturing and has decided to discontinue this product by June 2020 as it has been generated by the ascites method. We are sorry for any inconvenience this may cause.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer: Constituents: 100% PBS, 50% Glycerol
Purity: Ascites
Purification notes: Purified from ascites.
Clonality: Monoclonal
Clone number: 4A4
Isotype: IgG2b
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-density lipoprotein (LDL(ox)) possibly implicating the iNOS-S100A8/A9 transnitrosylase complex.

Cellular localization

Cytoplasm.

Form

Vimentin is found in connective tissue and in the cytoskeleton.

Applications

Our Abpromise guarantee covers the use of ab22651 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB</td>
<td>★★★☆☆☆☆</td>
<td>Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 57 kDa (predicted molecular weight: 54 kDa).</td>
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<tr>
<td>ELISA</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ICC/IF</td>
<td>★★★★☆☆☆☆</td>
<td>Use a concentration of 1 µg/ml.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 20436478</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td>★★★★☆☆☆☆</td>
<td>Use at an assay dependent concentration. PubMed: 20436478</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use 1µg for 10^6 cells. ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.</td>
</tr>
</tbody>
</table>

Target

Function

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Immunohistochemistry (Frozen sections) - Anti-Vimentin (phospho S55) antibody [4A4] (ab22651)

This image is courtesy of an anonymous Abreview

ab22651 staining Vimentin in Ferret brain tissue sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with paraformaldehyde, permeabilized with Triton-X and blocked with 7.5µg/ml 0.1 M glycine buffer for 30 minutes at room temperature. Samples were incubated with primary antibody (1/50) for 16 hours at 4°C. A Cy2®-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

Western blot - Anti-Vimentin (phospho S55) antibody [4A4] (ab22651)

All lanes:
Lane 1: U251 interphase cells
Lane 2: U251 M phase cells

Lysates/proteins at 5 µg/ml per lane.

Predicted band size: 54 kDa

Western Blot analysis using ab22651

ab22651 at 1µg/ml staining Vimentin (phospho S55) in human U251 cells by Immunocytochemistry/ Immunofluorescence.
Overlay histogram showing HeLa cells stained with ab22651 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween 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 (ab22651, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min) permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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