abcam

Product datasheet

# Anti-CD44 antibody [C44Mab-5] ab264539

KO VALIDATED Recombinant

1 References 8 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-CD44 antibody [C44Mab-5] |
| **Description** | Mouse monoclonal [C44Mab-5] to CD44 |
| **Host species** | Mouse |
| **Tested applications** | **Suitable for:** WB, Flow Cyt, IHC-P, IP |
| **Species reactivity** | **Reacts with:** Human |
| **Immunogen** | Tissue, cells or virus. This information is considered to be commercially sensitive. |
| **Positive control** | WB: MDA-MB-231 whole cell lysate. IHC-P: Human lung carcinoma and skin tissue. Flow: MDA-MB-231 cells IP: HAP1 cell lysate |
| **General notes** | This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**.<br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**. |

## Properties

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| **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: 7.2<br>Preservative: 0.01% Sodium azide<br>Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | C44Mab-5 |
| **Isotype** | IgG1 |

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|---|---|
| **Light chain type** | kappa |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab264539 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** | | Use a concentration of 1.226 µg/ml. Detects a band of approximately 82 kDa (predicted molecular weight: 81 kDa). |
| **Flow Cyt** | | Use a concentration of 1.055 µg/ml. |
| **IHC-P** | | Use a concentration of 0.253 µg/ml. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **IP** | | Use at an assay dependent concentration. |

## Target

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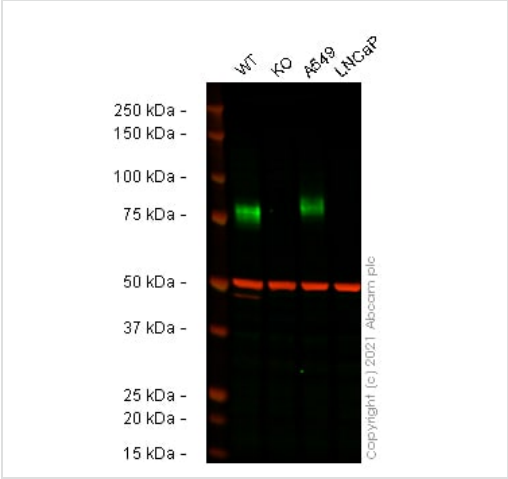
|---|---|
| **Function** | Receptor for hyaluronic acid (HA). Mediates cell-cell and cell-matrix interactions through its affinity for HA, and possibly also through its affinity for other ligands such as osteopontin, collagens, and matrix metalloproteinases (MMPs). Adhesion with HA plays an important role in cell migration, tumor growth and progression. Also involved in lymphocyte activation, recirculation and homing, and in hematopoiesis. Altered expression or dysfunction causes numerous pathogenic phenotypes. Great protein heterogeneity due to numerous alternative splicing and post-translational modification events. |
| **Tissue specificity** | Isoform 10 (epithelial isoform) is expressed by cells of epithelium and highly expressed by carcinomas. Expression is repressed in neuroblastoma cells. |
| **Sequence similarities** | Contains 1 Link domain. |
| **Domain** | The lectin-like LINK domain is responsible for hyaluronan binding. |
| **Post-translational modifications** | Proteolytically cleaved in the extracellular matrix by specific proteinases (possibly MMPs) in several cell lines and tumors.<br>N-glycosylated.<br>O-glycosylated; contains more-or-less-sulfated chondroitin sulfate glycans, whose number may affect the accessibility of specific proteinases to their cleavage site(s).<br>Phosphorylated; activation of PKC results in the dephosphorylation of Ser-706 (constitutive phosphorylation site), and the phosphorylation of Ser-672. |
| **Cellular localization** | Membrane. |

## Images




Western blot - Anti-CD44 antibody [C44Mab-5] (ab264539)

**All lanes :** Anti-CD44 antibody [C44Mab-5] (ab264539)

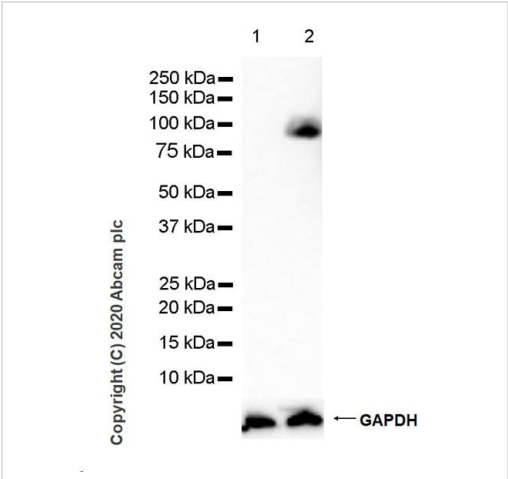
**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** CD44 knockout HeLa cell lysate
**Lane 3 :** A549 cell lysate
**Lane 4 :** LNCaP cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

**Predicted band size:** 81 kDa
**Observed band size:** 75-80 kDa

False colour image of Western blot: Anti-CD44 antibody [C44Mab-5] staining at 1.226 µg/ml, shown in green; Rabbit anti-alpha Tubulin antibody [EP1332Y] (**ab52866**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab264539 was shown to bind specifically to CD44. A band was observed at 75-80 kDa in wild-type HeLa cell lysates with no signal observed at this size in CD44 knockout cell line **ab262515** (knockout cell lysate **ab263938**). To generate this image, wild-type and CD44 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 Â°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.




Western blot - Anti-CD44 antibody [C44Mab-5] (ab264539)

**All lanes :** Anti-CD44 antibody [C44Mab-5] (ab264539) at 1.226 µg/ml

**Lane 1 :** MCF7 (human breast adenocarcinoma epithelial cell), whole cell lysate
**Lane 2 :** MDA-MB-231 (human breast adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

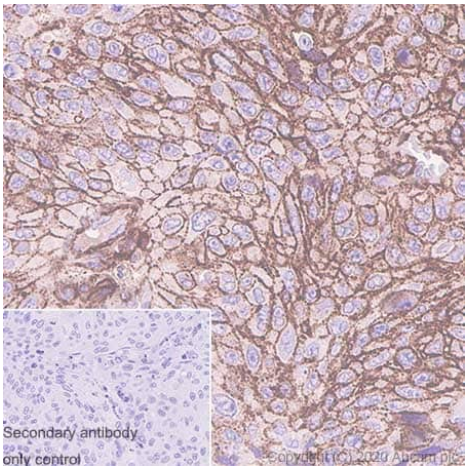
**Secondary**
**All lanes :** Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

**Predicted band size:** 81 kDa
**Observed band size:** 82 kDa

**Exposure time:** 70 seconds

Blocking/diluting buffer and concentration: 5% NFDM/TBST.

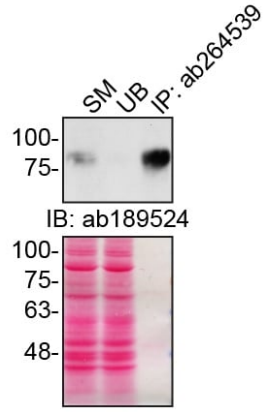
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab264539).




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD44 antibody [C44Mab-5] (ab264539)

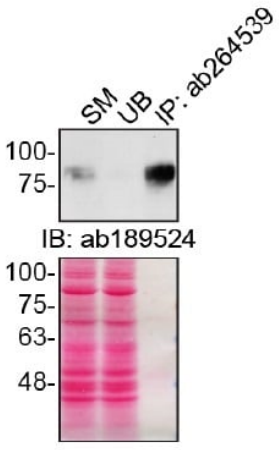
Formalin-fixed, paraffin-embedded Human lung carcinoma tissue stained for CD44 using ab264539 at 0.253 µg/mL followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) in immunohistochemical analysis. Counterstained with Hematoxylin. Membranous staining on Human lung carcinoma. The section was incubated with ab264539 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Secondary antibody only control: Used PBS instead of the primary antibody, secondary antibody was a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).



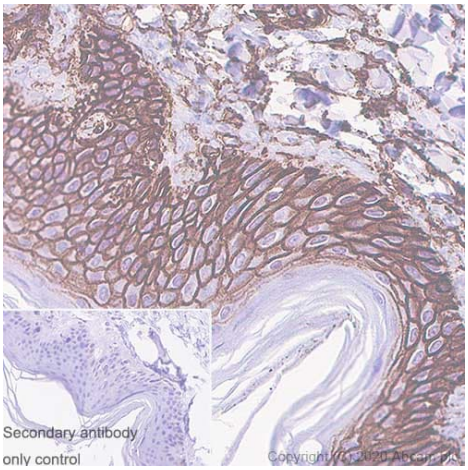

Immunoprecipitation - Anti-CD44 antibody [C44Mab-5] (ab264539)

Immunoprecipitation of CD44 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab264539 pre-coupled to prot.G-Sepharose beads. Samples were washed and processed for western blot with **ab189524** at 1/2000. This data was kindly provided by the YCharOS Inc., an open science company with the mission of characterizing every commercially available antibody reagent. Abcam are working with YCharOS to support their mission of antibody characterisation using knock out cell lines.




Immunoprecipitation - Anti-CD44 antibody [C44Mab-5] (ab264539)

Immunoprecipitation of CD44 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab264539 pre-coupled to prot.G-Sepharose beads. Samples were washed and processed for western blot with **ab189524** at 1/2000. SM=10% starting material; UB=10% unbound fraction; IP=immunoprecipitate. These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.

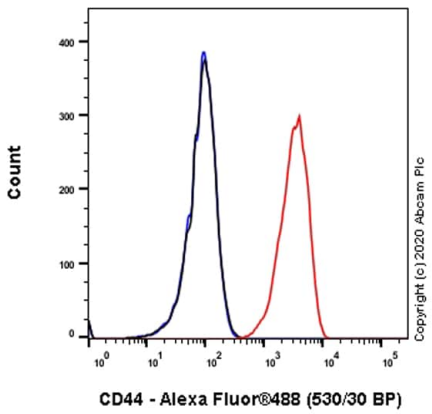



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD44 antibody [C44Mab-5] (ab264539)

Formalin-fixed, paraffin-embedded Human skin tissue stained for CD44 using ab264539 at 0.253 µg/mL followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) in immunohistochemical analysis. Counterstained with Hematoxylin. Membranous staining on human skin. The section was incubated with ab264539 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

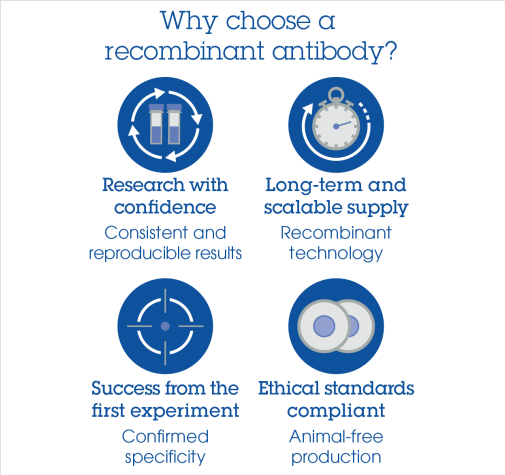
Secondary antibody only control: Used PBS instead of the primary antibody, secondary antibody was a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).




Flow Cytometry - Anti-CD44 antibody [C44Mab-5] (ab264539)

Flow cytometric analysis of MDA-MB-231 (Human breast adenocarcinoma epithelial cell) cell line labeling CD44 (Red) using ab264539 at 1.055 µg/mL followed by Goat anti mouse IgG (Alexa Fluor® 488, **ab150113**) at 1/2000 dilution. Mouse monoclonal IgG was used as the isotype control (Black). Cells without incubation with primary antibody and secondary antibody (Blue).

Gated on viable cells.




Anti-CD44 antibody [C44Mab-5] (ab264539)

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