abcam

Product datasheet

Anti-CD44 antibody [C44Mab-5] - BSA and Azide free ab264546



リコンピナント

画像数7

製品の概要

製品名 Anti-CD44 antibody [C44Mab-5] - BSA and Azide free

製品の詳細 Mouse monoclonal [C44Mab-5] to CD44 - BSA and Azide free

由来種 Mouse

アプリケーション 適用あり: WB, IHC-P, Flow Cyt, IP

種交差性 交差種: Human

免疫原 Tissue, cells or virus. This information is considered to be commercially sensitive.

ポジティブ・コントロール WB: MDA-MB-231 whole cell lysate. IHC-P: Human lung carcinoma and skin tissue. Flow: MDA-

MB-231 cells IP: HAP1 cell lysate.

特記事項 ab264546 is the carrier-free version of ab264539.

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

バッファー pH: 7.2

1

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 C44Mab-5

アイソタイプ lgG1 軽鎖の種類 kappa

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab264546の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Detects a band of approximately 82 kDa (predicted molecular weight: 81 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

ターゲット情報

機能 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.

組織特異性 soform 10 (epithelial isoform) is expressed by cells of epithelium and highly expressed by

carcinomas. Expression is repressed in neuroblastoma cells.

配列類似性 Contains 1 Link domain.

ドメイン The lectin-like LINK domain is responsible for hyaluronan binding.

翻訳後修飾 Proteolytically cleaved in the extracellular matrix by specific proteinases (possibly MMPs) in

several cell lines and tumors.

N-glycosylated.

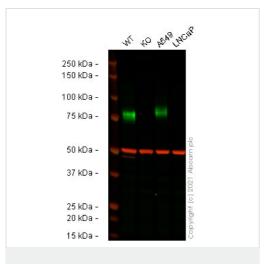
O-glycosylated; contains more-or-less-sulfated chondroitin sulfate glycans, whose number may

affect the accessibility of specific proteinases to their cleavage site(s).

Phosphorylated; activation of PKC results in the dephosphorylation of Ser-706 (constitutive

phosphorylation site), and the phosphorylation of Ser-672.

画像



Western blot - Anti-CD44 antibody [C44Mab-5] - BSA and Azide free (ab264546)

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

Lane 1: Wild-type HeLa cell lysate

Lane 2: CD44 knockout HeLa cell lysate

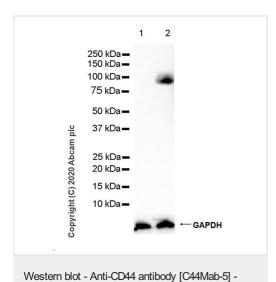
Lane 3 : A549 cell lysate
Lane 4 : LNCaP cell lysate

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 °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.



BSA and Azide free (ab264546)

All lanes : Anti-CD44 antibody [C44Mab-5] ($\underline{ab264539}$) at 1.226 $\mu g/ml$

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

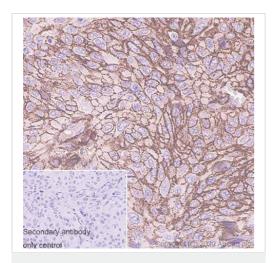
All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

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

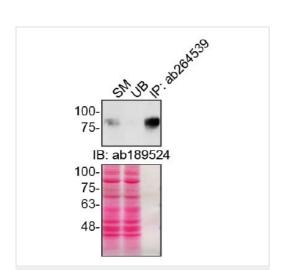
Exposure time: 70 seconds

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 (<u>ab264539</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [C44Mab-5] - BSA and Azide free (ab264546)



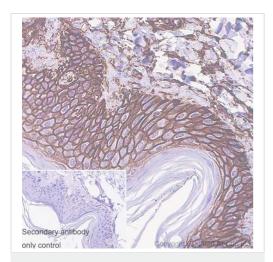
Immunoprecipitation - Anti-CD44 antibody [C44Mab-5] - BSA and Azide free (ab264546)

Formalin-fixed, paraffin-embedded Human lung carcinoma tissue stained for CD44 using <u>ab264539</u> 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 <u>ab264539</u> 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.

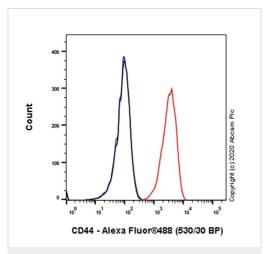
Secondary antibody only control: Used PBS instead of the primary antibody, secondary antibody was a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection).

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

This data was developed using the same antibody clone in a different buffer formulation (ab264546). Immunoprecipitation of CD44 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of ab264539 precoupled 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 paraffinembedded sections) - Anti-CD44 antibody [C44Mab-5] - BSA and Azide free (ab264546)



Flow Cytometry - Anti-CD44 antibody [C44Mab-5] - BSA and Azide free (ab264546)

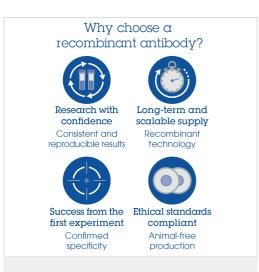
Formalin-fixed, paraffin-embedded Human skin tissue stained for CD44 using <u>ab264539</u> at 0.253 µg/mL followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection) in immunohistochemical analysis. Counterstained with Hematoxylin. Membranous staining on human skin. The section was incubated with <u>ab264539</u> 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).

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

Flow cytometric analysis of MDA-MB-231 (Human breast adenocarcinoma epithelial cell) cell line labeling CD44 (Red) using ${\tt ab264539}$ at 1.055 µg/mL followed by Goat anti mouse lgG (Alexa Fluor 488, ${\tt ab150113}$) at 1/2000 dilution. Mouse monoclonal lgG was used as the isotype control (Black). Cell without incubation with primary antibody and secondary antibody (Blue). Gated ov viable cells.

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



Anti-CD44 antibody [C44Mab-5] - BSA and Azide free (ab264546)

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