abcam

Product datasheet

Anti-CD44 antibody [EPR18668] ab189524

敲除 验证 重组 RabMAb

<u>59 References</u> 18 图像

概述

产品名称	Anti-CD44 抗体 [EPR18668]
描述	兔 单 克隆抗体[EPR18668] to CD44
宿主	Rabbit
经测试应 用	适用于: WB, IHC-P, IP 不适用于: Flow Cyt or ICC/IF
种属反 应性	与反应: Mouse, Rat, Human
免疫原	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
阳性 对 照	WB: Human fetal brain, fetal heart, fetal kidney and fetal spleen lysates; Human thymus and skin lysates; HAP1, HeLa, A549, U-87 MG, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Mouse brain and spleen lysates; Rat brain, heart, kidney and spleen lysates; IHC-P: Human breast, kidney, endometrial cancer, tonsil and breast cancer tissues; Mouse colon, stomach and spleen tissues; Rat stomach and spleen tissues; IP: A549 whole cell lysate.
常 规说 明	 This product is a recombinant monoclonal antibody, which offers several advantages including: High batch-to-batch consistency and reproducibility Improved sensitivity and specificity Long-term security of supply Animal-free production For more information see here. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>.

性能	
形式	Liquid
存 放 说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
纯 度	Protein A purified

克隆	单 克隆
克隆 编号	EPR18668
同种型	lgG

应用

The Abpromise guarantee

Abpromise™承诺保证使用ab189524于以下的经测试应用

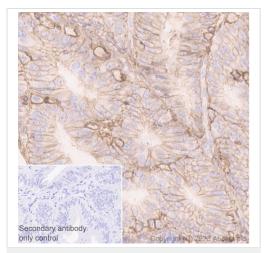
"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应 用	Ab评论	说明
WB		1/1000. Detects a band of approximately 82 kDa (predicted molecular weight: 82 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

应用说明

Is unsuitable for Flow Cyt or ICC/IF.

靶 标					
功能	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.				
组织 特异性	lsoform 10 (epithelial isoform) is expressed by cells of epithelium and highly expressed by carcinomas. Expression is repressed in neuroblastoma cells.				
序列相似性	Contains 1 Link domain.				
结 构域	The lectin-like LINK domain is responsible for hyaluronan binding.				
翻 译 后修 饰	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.				
细 胞定位	Membrane.				

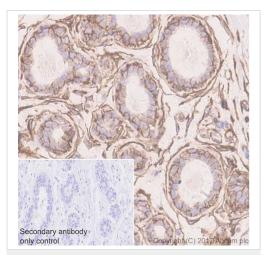


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue labeling CD44 with ab189524 at 1/8000 dilution (0.099 µg/ml), followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Positive staining on human endometrial carcinoma. The section was incubated with ab189524 at 4°C overnight.Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

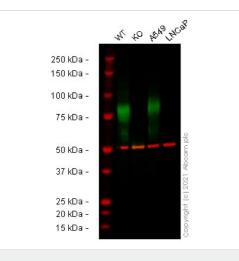
Perform heat mediated antigen retrieval with Tris/EDTA buffer (ab93684) pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524) Immunohistochemical analysis of paraffin-embedded human breast tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on human breast [PMID: 20103682].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



Western blot - Anti-CD44 antibody [EPR18668] (ab189524) All lanes : Anti-CD44 antibody [EPR18668] (ab189524) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : CD44 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

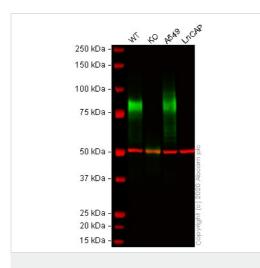
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate Lane 4 : LNCaP (Human prostate cancer cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 82 kDa Observed band size: 70-85 kDa

False colour image of Western blot: Anti-CD44 antibody [EPR18668] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab189524 was shown to bind specifically to CD44. A band was observed at 70-85 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-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-CD44 antibody [EPR18668] (ab189524)

All lanes : Anti-CD44 antibody [EPR18668] (ab189524) at 1/1000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : CD44 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : LNCaP (Human prostate cancer cell line) whole cell lysate

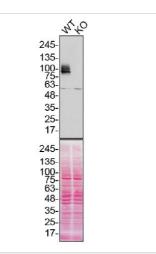
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 82 kDa Observed band size: 80 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab189524 observed at 80 kDa. Red - loading control, <u>ab7291</u> (Mouse anti-Alpha Tubulin [DM1A]) observed at 55kDa.

ab189524 was shown to react with CD44 in wild-type HeLa cells in western blot. Loss of signal was observed when CD44 knockout sample was used. Wild-type HeLa and CD44 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween[®]) before incubation with ab189524 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-CD44 antibody [EPR18668] (ab189524) All lanes : Anti-CD44 antibody [EPR18668] (ab189524)

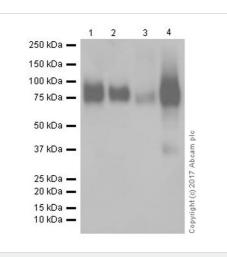
Lane 1 : Wild-type HAP1 cell lysate Lane 2 : CD44 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 82 kDa

ab189524 was shown to react with CD44 in wild-type HAP1 cells in Western blot with loss of signal observed in a CD44 knockout cell line. Wild-type HAP1 and CD44 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab189524 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at 0.2ug/mL before imaging. 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.



Western blot - Anti-CD44 antibody [EPR18668] (ab189524) All lanes : Anti-CD44 antibody [EPR18668] (ab189524) at 1/1000 dilution

- Lane 1 : Human fetal brain lysate Lane 2 : Human fetal heart lysate Lane 3 : Human fetal kidney lysate
- Lane 4 : Human fetal spleen lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/10000 dilution

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

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

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Western blot - Anti-CD44 antibody [EPR18668] (ab189524) All lanes : Anti-CD44 antibody [EPR18668] (ab189524) at 1/2000 dilution

Lane 1 : Human thymus lysate

Lane 2 : Human skin lysate

Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate Lane 4 : U-87 MG (Human glioblastoma-astrocytoma epithelial cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

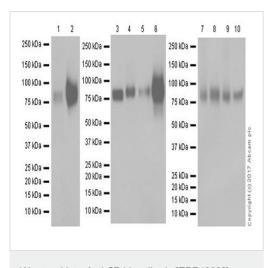
Lanes 1-2 : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/10000 dilution Lanes 3-4 : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

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

Exposure time: 1 second

Blocking/Dilution buffer: 5% NFDM/TBST.

Due to high sequence homology between CD44 isoforms, within the immunogen region, this antibody cross reacts with most isoforms of CD44. The staining pattern is consistent with reference (PMID 19507218).



Western blot - Anti-CD44 antibody [EPR18668] (ab189524) Lanes 1-6 : Anti-CD44 antibody [EPR18668] (ab189524) at 1/1000 dilution

Lanes 7-10 : Anti-CD44 antibody [EPR18668] (ab189524) at 1/20000 dilution

Lane 1 : Mouse brain lysate at 10 µg

Lane 2 : Mouse spleen lysate at 10 µg

Lane 3 : Rat brain lysate at 10 μg

Lane 4 : Rat heart lysate at 10 μ g

Lane 5 : Rat kidney lysate at 10 μg

Lane 6 : Rat spleen lysate at 10 μg

Lane 7 : C6 (Rat glial tumor cell line) whole cell lysate

Lane 8 : RAW 264.7 (Mouse macrophage cell line transformed

with Abelson murine leukemia virus) whole cell lysate

Lane 9 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 10 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

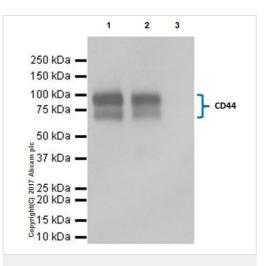
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

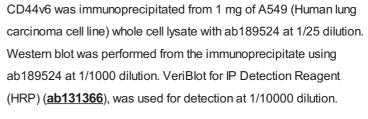
Predicted band size: 82 kDa Observed band size: 82 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1, 2, 3, 4, 5 and 6: 5 seconds; Lane 7, 8, 9 and 10: 1 second.





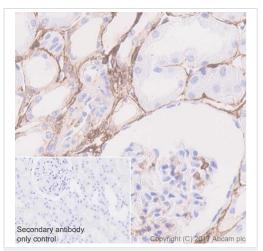


Lane 1: A549 whole cell lysate 10 µg (Input).

Lane 2: ab189524 IP in A549 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab189524 in A549 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 1 second.

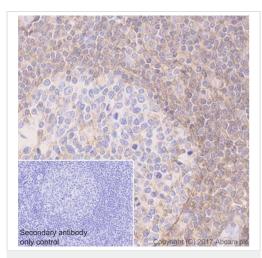


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on human kidney.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



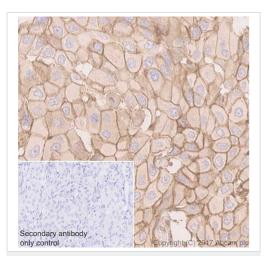
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded human tonsil tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on human tonsil.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

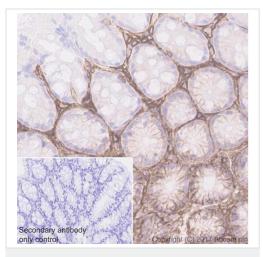


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded human breast cancer tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on human breast cancer [PMID: 15867228].

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



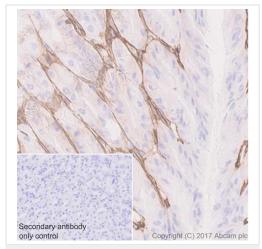
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on mouse colon.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

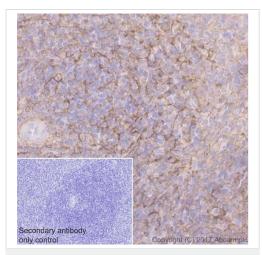


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded mouse stomach tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on mouse stomach.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



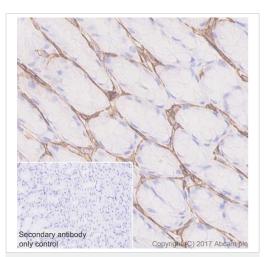
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded mouse spleen tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on mouse spleen.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

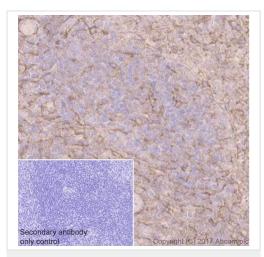


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)

Immunohistochemical analysis of paraffin-embedded rat stomach tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous staining on rat stomach.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [EPR18668] (ab189524)



Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling CD44 with ab189524 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous and weak cytoplasmic staining on rat spleen.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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