abcam

Product datasheet

Anti-CD44 antibody [SP37] ab101531

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-CD44 antibody [SP37]
Description	Rabbit monoclonal [SP37] to CD44
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Cow, Dog
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human esophageal carcinoma tissue. WB: HeLa and A549 cell lysates.
General notes	**This product is FOR RESEARCH USE ONLY. For commercial use, please contact partnerships@abcam.com.**

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.60 Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA
Purity	Protein A/G purified
Purification notes	Purified from TCS by protein A/G.
Clonality	Monoclonal
Clone number	SP37
Isotype	IgG

Applications

The Abpromise guarantee	Our **Abpromise guarantee** covers the use of ab101531 in the following tested applications.

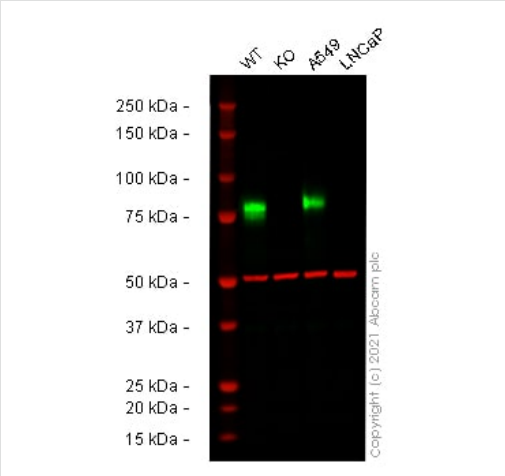
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/100. Boil tissue section in citrate buffer pH 6.0 for 10 minutes followed by cooling at room temperature for 20 minutes. Incubate with primary antibody for 30 minutes at room temperature.
WB		Use at an assay dependent concentration. Predicted molecular weight: 80 kDa.

Target

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. 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.
Cellular localization	Membrane.

Images



Western blot - Anti-CD44 antibody [SP37] (ab101531)

All lanes : Anti-CD44 antibody [SP37] (ab101531) at 1/1000 dilution

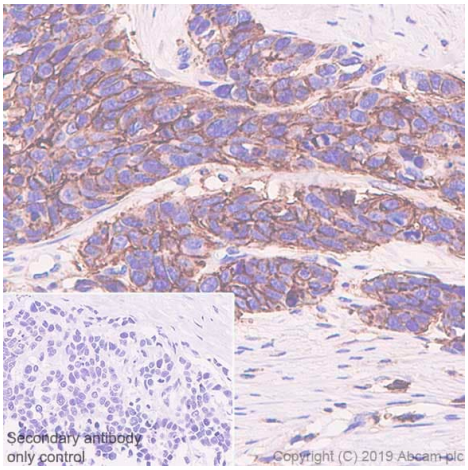
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CD44 knockout HeLa cell lysate
Lane 3 : A549 cell lysate
Lane 4 : LNCaP cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

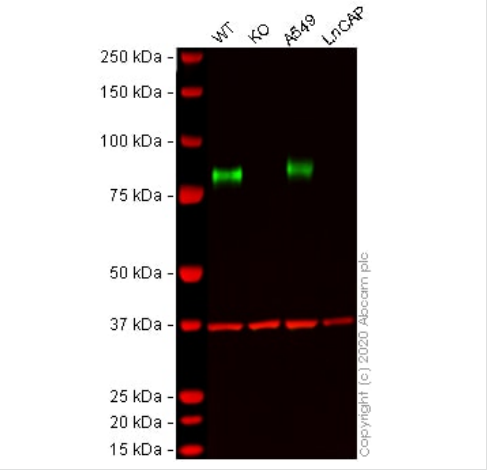
Predicted band size: 80 kDa
Observed band size: 75-80 kDa

False colour image of Western blot: Anti-CD44 antibody [SP37] 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, ab101531 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD44 antibody [SP37] (ab101531)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human esophageal carcinoma tissue sections labeling CD44 with Purified ab101531 at 1/100 dilution (1.03 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with sodium citrate buffer (pH 6.0, epitope retrieval solution 1) for 20mins. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-CD44 antibody [SP37] (ab101531)

All lanes : Anti-CD44 antibody [SP37] (ab101531) 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.

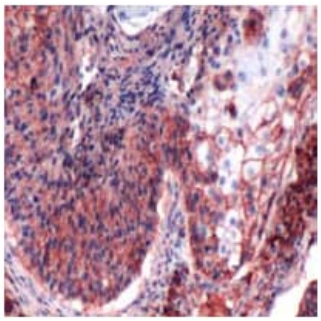
Performed under reducing conditions.

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

Lanes 1 - 4: Merged signal (red and green). Green - ab101531 observed at 80 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

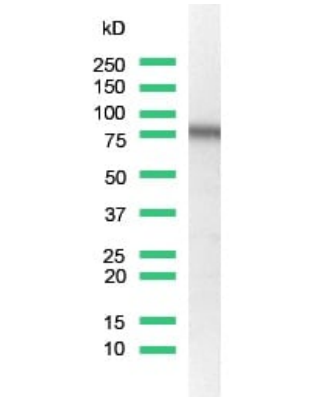
ab101531 was shown to react with CD44 in wild-type HeLa cells in western blot. Loss of signal was observed when CD44 knockout sample was used. Wild-type HeLa and CD44 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab101531 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD44 antibody [SP37] (ab101531)

Formalin-fixed, paraffin-embedded human esophageal carcinoma tissue stained for CD44 with ab101531 at a 1/100 dilution in immunohistochemical analysis.



Western blot - Anti-CD44 antibody [SP37] (ab101531)

Anti-CD44 antibody [SP37] (ab101531) at 1/25 dilution + HeLa cell lysate

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

Anti-CD44 antibody [SP37] (ab101531)

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