abcam

Product datasheet

Anti-CD44 antibody [SP37] - BSA and Azide free ab236436



Recombinant

RabMAb

5 Images

Overview

Product name Anti-CD44 antibody [SP37] - BSA and Azide free

Description Rabbit monoclonal [SP37] to CD44 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: IHC-P, WB

Species reactivity Reacts with: Human

Predicted to work with: Cow, Dog

Immunogen Synthetic peptide within Human CD44 aa 150-250 (internal sequence). The exact immunogen

sequence used to generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact**

our Scientific Support team to discuss your requirements.

Database link: P16070-1

Positive control IHC-P: Human esophageal carcinoma tissue. WB: HeLa and A549 cell lysates.

General notes ab236436 is the carrier-free version of <u>ab101531</u>.

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.

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

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- Animal-free production

For more information see here.

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. Do Not Freeze.

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

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 quarantee covers the use of ab236436 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		Use at an assay dependent concentration. 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. Detects a band of approximately 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

lsoform 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 Proteolytically cleaved in the extracellular matrix by specific proteinases (possibly MMPs) in

several cell lines and tumors.

N-glycosylated.

 $\hbox{O-glycosylated; contains more-or-less-sulfated chondroit in sulfate glycans, whose number \ may}$

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

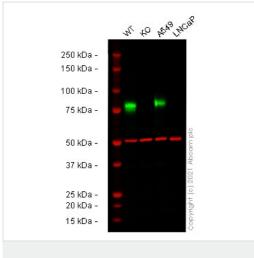
Phosphorylated; activation of PKC results in the dephosphorylation of Ser-706 (constitutive

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

Cellular localization Membrane.

Images

modifications



Western blot - Anti-CD44 antibody [SP37] - BSA and Azide free (ab236436)

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

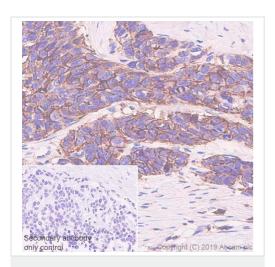
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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°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 lgG H&L

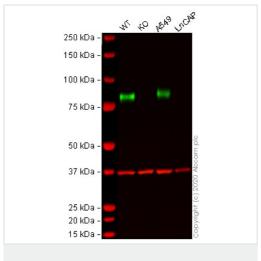
(IRDye $^{\$}$ 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye $^{\$}$ 680RD) preabsorbed (<u>ab216776</u>) at 1/20000 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [SP37] - BSA and Azide free (ab236436)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab101531</u>)



Western blot - Anti-CD44 antibody [SP37] - BSA and Azide free (ab236436)

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.

Observed band size: 80 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab101531</u>).

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

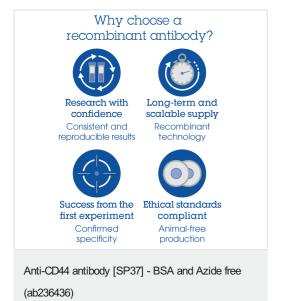
ab101531 was shown to react with CD44 in wild-type HeLa cells in

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 <u>ab101531</u> and <u>ab8245</u> (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 lgG H&L (IRDye[®] 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-CD44 antibody [SP37] - BSA and Azide free (ab236436)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (ab101531).



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