abcam

Product datasheet

Anti-CD44 antibody [Hermes-3] - BSA and Azide free ab255946

KO VALIDATED Recombinant

★★★★★ 1 Abreviews 10 Images

Overview

Product name	Anti-CD44 antibody [Hermes-3] - BSA and Azide free
Description	Mouse monoclonal [Hermes-3] to CD44 - BSA and Azide free
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, IP, ELISA, IHC-P, WB
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant fragment corresponding to Human CD44. Run BLAST with ExPASy Run BLAST with NCBI
Positive control	WB: HAP1, A549 and HeLa whole cell lysate. IHC-P: Human skin tissue. Human bladder carcinoma tissue. IP: HAP1 cell lysate. ICC/IF: HAP1, wild-type HeLa cells.
General notes	ab255946 is the carrier-free version of **ab254530**. 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 **carrier-free** 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 **conjugation kits** 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 - Animal-free production

For more information **see here**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Hermes-3
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255946 in the following tested applications.

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

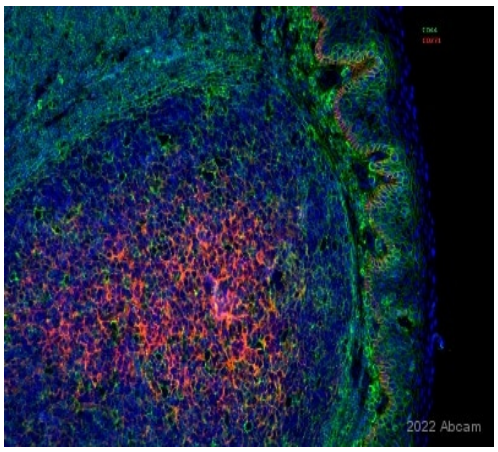
Application	Abreviews	Notes
ICC/IF		1/500.
IP		Use at an assay dependent concentration.
ELISA		Use 100-1000µg for 10 cells. 100-1000 ng/ml.
IHC-P	★★★★★ (1)	Use a concentration of 0.107 µg/ml. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use a concentration of 0.536 µg/ml. Predicted molecular weight: 81 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.

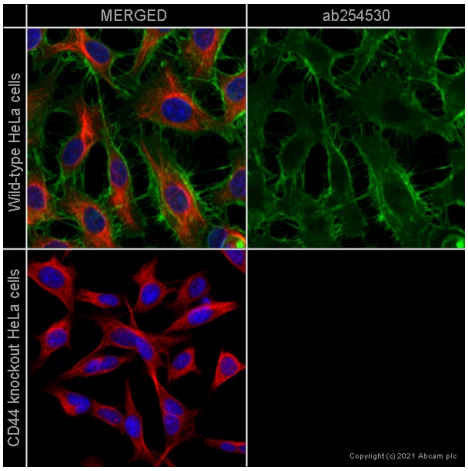
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

This image is courtesy of an Abreview submitted by Natalie Papazian

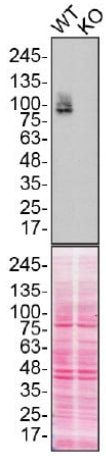
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue staining CD44 with ab255946 at 1/200 dilution and co-stained with **ab256584**. Secondary antibody used was Alexa Fluor® 488 Donkey anti-mouse IgG (H+L)at 1/200 dilution. The tissue was incubated for 18 hours with PBS +2% normal human serum at 4°C. Blocking was done with 5% serum for 1 hour at room temperature. Heat mediated antigen retrieval with Tris-EDTA 1mM PH9.



Immunocytochemistry/ Immunofluorescence - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

This data was developed using the same antibody clone in a different buffer formulation (**ab254530**). **ab254530** staining CD44 in wild-type HeLa cells (top panel) and CD44 knockout HeLa cells (**ab262515**)(bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Tween for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab254530** at 0.4µg/ml concentration and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems

TCS SP8).



Western blot - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

All lanes : Anti-CD44 antibody [Hermes-3] (**ab254530**) at 1/1000 dilution

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

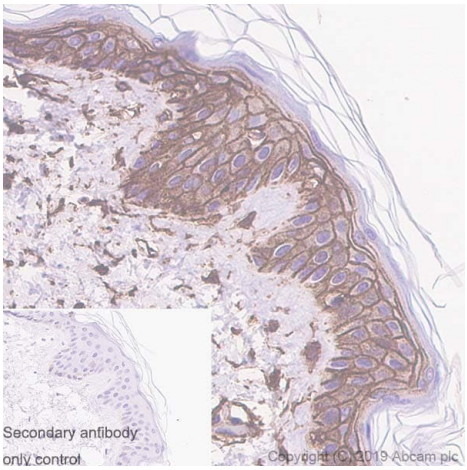
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 81 kDa

This data was developed using the same antibody in a different buffer formulation (**ab254530**).

ab254530 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 **ab254530** overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-mouse HRP secondary antibodies at 0.3ug/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.

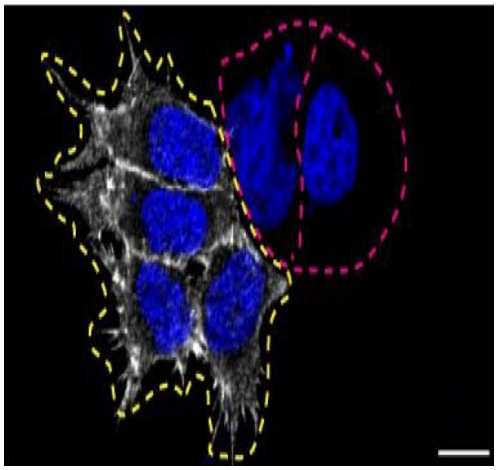


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, Glycerol and sodium azide (**ab254530**).

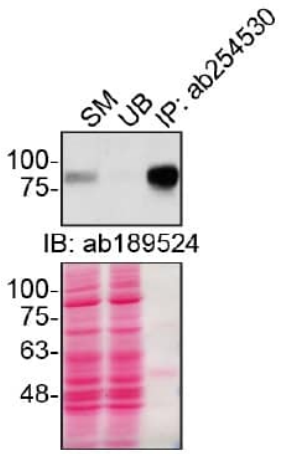
Immunohistochemical analysis of paraffin-embedded human skin tissue labeling CD44 with **ab254530** at 0.107µg/ml, followed by ready to use secondary. Membranous staining on human skin is observed. The section was incubated with **ab254530** for 5 mins at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, followed by ready to use secondary.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



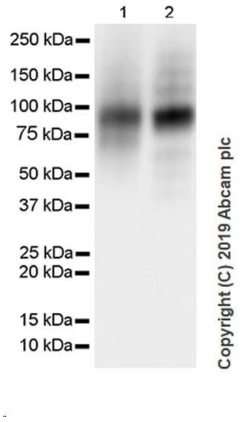
Immunocytochemistry/ Immunofluorescence - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

This data was developed using the same antibody clone in a different buffer formulation (**ab254530**). **ab254530** was shown to react with CD44 in wild-type HAP1 cells in Immunocytochemistry with loss of signal observed in a CD44 knockout cell line. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1/2000. The cells were then incubated with **ab254530** at 1/500 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880). 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.



Immunoprecipitation - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

This data was developed using the same antibody clone in a different buffer formulation (ab255946). Immunoprecipitation of CD44 in HAP1 cells. Lysates were prepared and immunoprecipitation was performed using 1.0 µg of **ab254530** 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.



Western blot - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

All lanes : Anti-CD44 antibody [Hermes-3] (**ab254530**) at 0.536 µg/ml

Lane 1 : A549 (human lung carcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

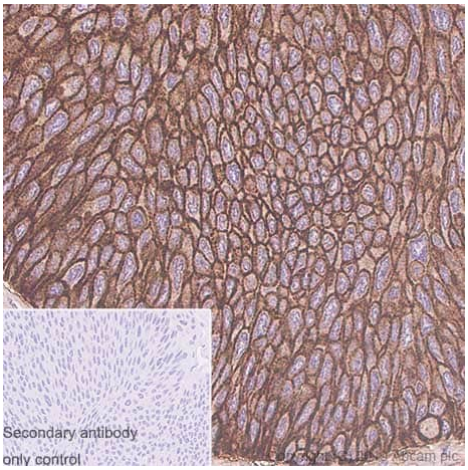
Secondary
All lanes : Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10000 dilution

Predicted band size: 81 kDa

Exposure time: 1 second

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, Glycerol and sodium azide (**ab254530**).

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

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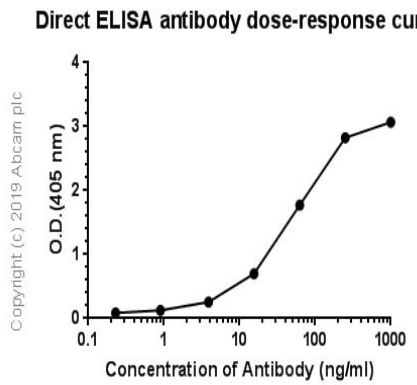
Immunohistochemical analysis of paraffin-embedded human bladder carcinoma tissue labeling CD44 with **ab254530** at 0.107µg/ml, followed by ready to use secondary. Membranous staining on human bladder carcinoma is observed. The section was incubated with **ab254530** for 5 mins at RT. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, followed by ready to use secondary.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



ELISA - Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

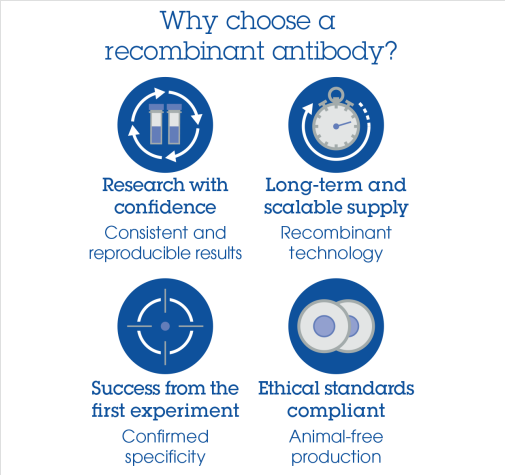
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, Glycerol and sodium azide (**ab254530**).

ELISA - Anti-CD44 antibody [Hermes-3] (**ab254530**).

Antigen: Human CD44.

ab254530 used at 1000-0 ng/ml.

Secondary is an Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L) used at a 1/1000 dilution.



Anti-CD44 antibody [Hermes-3] - BSA and Azide free (ab255946)

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