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Product datasheet

Anti-CD44 antibody [MEM-263] ab9524

KO VALIDATED

7 References 5 Images

Overview

Product name	Anti-CD44 antibody [MEM-263]
Description	Mouse monoclonal [MEM-263] to CD44
Host species	Mouse
Tested applications	**Suitable for:** WB, Flow Cyt, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to African green monkey CD44. COS-7 cells Database link: **A0A0D9QZF5**
Epitope	extracellular (N-terminal) domain
Positive control	Flow Cyt: Human peripheral blood lymphocytes. IHC-P: Human Skin Melanoma. WB: HPB-ALL, HeLa, MOLT-4 and A549 cell lysates.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Protein A purified
Purification notes	Purified from TCS. Purity >95% by SDS-PAGE.
Clonality	Monoclonal

Clone number	MEM-263
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab9524 in the following tested applications.

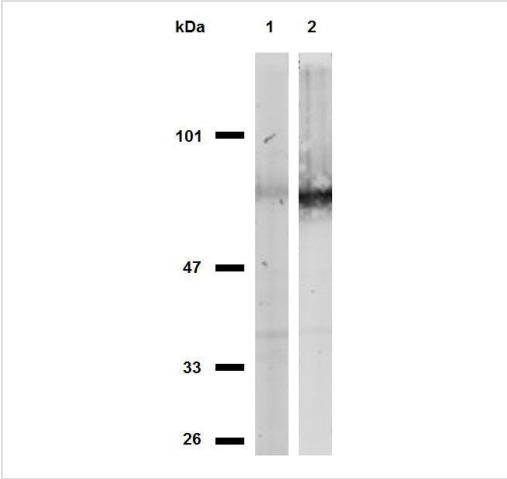
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use a concentration of 2 µg/ml. Use under non reducing condition.
Flow Cyt		Use a concentration of 4 µg/ml. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P		Use a concentration of 10 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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

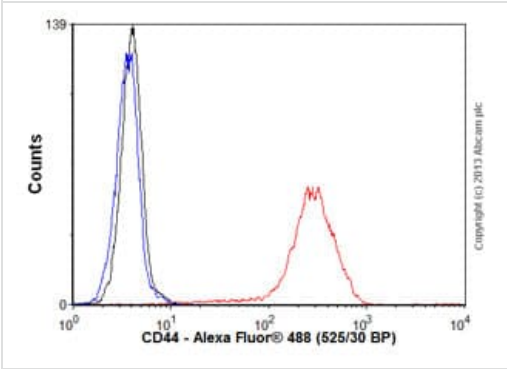


Western blot - Anti-CD44 antibody [MEM-263] (ab9524)

All lanes : Anti-CD44 antibody [MEM-263] (ab9524) at 2 µg/ml

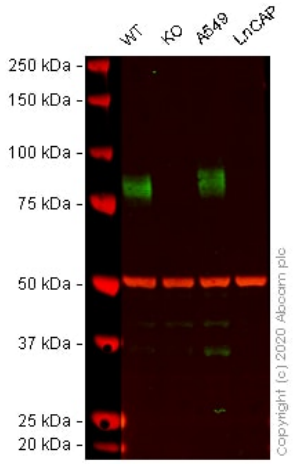
Lane 1 : MOLT-4 cells (human T lymphoblast; acute lymphoblastic leukemia)
Lane 2 : HeLa cells (human epithelial cell line from cervix adenocarcinoma)

Performed under non-reducing conditions.



Flow Cytometry - Anti-CD44 antibody [MEM-263] (ab9524)

Human peripheral blood lymphocytes stained with ab9524 (red line). Human whole blood was processed using a modified protocol based on Chow *et al*, 2005 (PMID: 16080188). In brief, human whole blood was fixed in 4% formaldehyde (methanol-free) for 10 min at 22°C. Red blood cells were then lyzed by the addition of Triton X-100 (final concentration - 0.1%) for 15 min at 37°C. For experimentation, cells were treated with 50% methanol (-20°C) for 15 min at 4°C. Cells were then incubated with the antibody (ab9524, 0.1µg/1x10^6 cells) for 30 min at 4°C. The secondary antibody used was Alexa Fluor® 488 goat anti-mouse IgG (H&L) (**ab150113**) at 1/2000 dilution for 30 min at 4°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >30,000 total events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. Gating strategy - peripheral blood lymphocytes.



Western blot - Anti-CD44 antibody [MEM-263] (ab9524)

All lanes : Anti-CD44 antibody [MEM-263] (ab9524) at 2 µg/ml

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

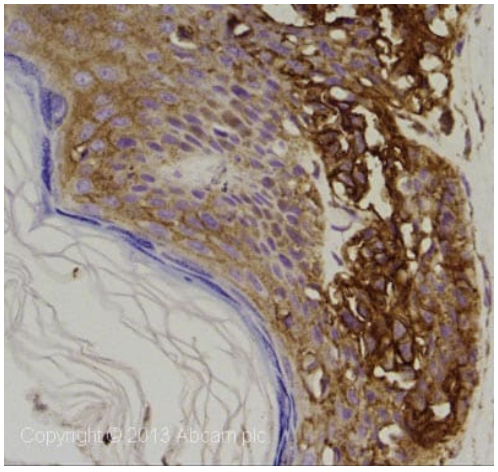
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 80 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab9524 observed at 80 kDa. Red - loading control, **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

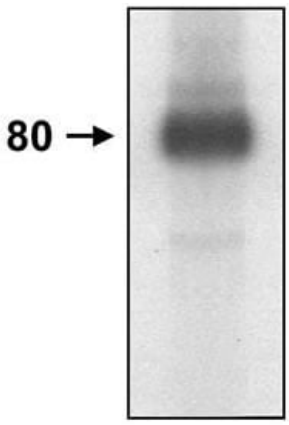
ab9524 was shown to react with CD44 in wild-type HeLa cells in western blot. Loss of signal was observed when CD44 knockout sample was used. Wild-type and CD44 knockout HeLa cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab9524 and **ab52866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at 2 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) 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 [MEM-263] (ab9524)

IHC image of CD44 staining in Human Skin Melanoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab9524, 0.5 µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Western blot - Anti-CD44 antibody [MEM-263] (ab9524)

Western blotting of HPB-ALL cell lysate (non-reduced sample) stained by ab9524.

Western blotting of HPB-ALL cell lysate (non-reduced sample) stained by ab9524.

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