Human CD44s Pan Specific Antibody

Monoclonal Mouse IgG_{2A} Clone # 2C5

Catalog Number: BBA10

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD44s on a panel of CD44 transfected COS cells by flow cytometry (Fox, S.B. *et al.* (1994) Cancer Res. **54:**4539). This antibody recognizes an epitope in the invariant N-terminal region of all CD44 protein isoforms.
Source	Monoclonal Mouse IgG_{2A} Clone # 2C5
Purification	Protein A or G purified from ascites
Immunogen	Recombinant human CD44v3-10 (includes the invariant N-terminal exons and CD44v3-10 exons)
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose.

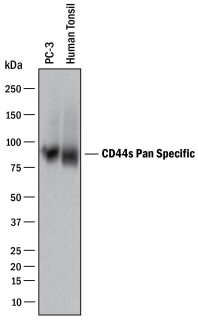
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	2 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	Human whole blood monocytes & MDA-MB-231 human breast cancer cell line lysates
Immunohistochemistry	8-25 µg/mL	See Below
Simple Western	20 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
Knockout Validated	CD44 is specifically detected in MDA-MB-231 parental cell line but is not detectable in knockout MDA-MB-231 cell line.	
Immunoprecipitation	Fox, S.B. *et al.* (1994) Cancer Res. **54**:4539.	

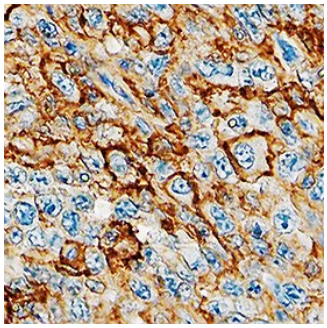
DATA

Western Blot



Detection of Human CD44 by Western Blot. Western blot shows lysates of PC-3 human prostate cancer cell line and human tonsil tissue. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human CD44 s Pan Specific Monoclonal Antibody (Catalog # BBA10) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for CD44 at approximately 90 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



CD44 in Human Lymphoma. CD44 was detected in immersion fixed paraffin-embedded sections of human lymphoma using Mouse Anti-Human CD44 s Pan Specific Monoclonal Antibody (Catalog # BBA10) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to plasma membrane. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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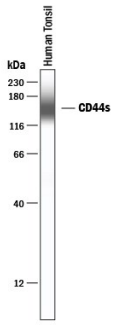
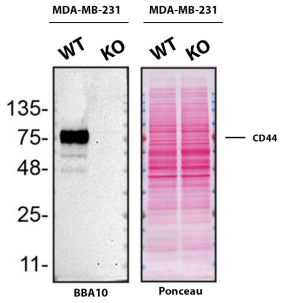
Simple Western



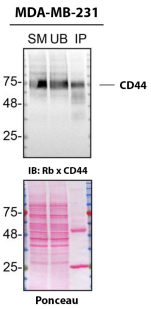
Detection of Human CD44 by Simple Western™. Simple Western lane view shows lysates of human tonsil tissue, loaded at 0.2 mg/mL. A specific band was detected for CD44 at approximately 156 kDa (as indicated) using 20 µg/mL of Mouse Anti-Human CD44 s Pan Specific Monoclonal Antibody (Catalog # BBA10) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Knockout Validated



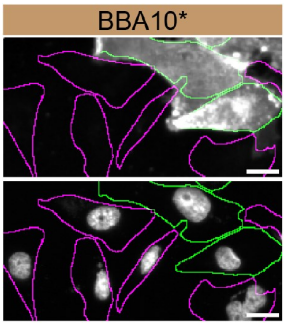
Western Blot Shows CD44 Specificity Using Knockout Cell Line. Western blot shows lysates of MDA-MB-231 human breast cancer parental cell line and CD44 knockout MDA-MB-231 cell line (KO). Nitrocellulose membrane was probed with Mouse Anti-Human CD44 s Pan Specific Monoclonal Antibody (Catalog # BBA10) followed by HRP-conjugated secondary antibody. A specific band was detected for CD44 at approximately 81.5 kDa (as indicated) in the parental MDA-MB-231 cell line, but is not detectable in knockout MDA-MB-231 cell line. Primary antibody concentration used: 2.5 µg/mL. The Ponceau stained transfer of the blot is shown. This experiment was conducted under reducing conditions. Image, protocol, and testing courtesy of YCharOS Inc. See ycharos.com for additional details.

Immunoprecipitation



Detection of CD44 by Immunoprecipitation. MDA-MB-231 human breast cancer cell line lysates were prepared and immunoprecipitation was performed using 2.0 µg of Mouse Anti-Human CD44 s Pan Specific Monoclonal Antibody (Catalog # BBA10) pre-coupled to Dynabeads Protein G. Immunoprecipitated CD44 was detected in Western Blot with a rabbit CD44 antibody used at 1/3000. The Ponceau stained transfer of the blot is shown. SM=4% starting material; UB=4% unbound fraction; IP=immunoprecipitate; HC=antibody heavy chain. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).

Knockout Validated



CD44 Specificity is Shown by Immunocytochemistry in Knockout Cell Line. MDA-MB-231 human breast cancer parental cell line WT and CD44 MDA-MB-231 KO cells were labelled with a green or a far-red fluorescent dye, respectively. Cells were stained with Mouse Anti-Human CD44 s Pan Specific Monoclonal Antibody (Catalog # BBA10) followed by incubation with an Alexa-fluor 555 conjugated secondary antibody (upper panel). DAPI-only counterstained cells shown on a lower panel. Acquisition of the blue (nucleus-DAPI), green (identification of WT cells), red (antibody staining) and far-red (identification of KO cells) channels was performed. Representative images of the blue and red (grayscale) channels are shown. WT and KO cells are outlined with green and magenta dashed line, respectively. Primary antibody concentration used: 2 µg/mL. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).

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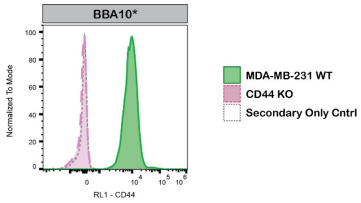
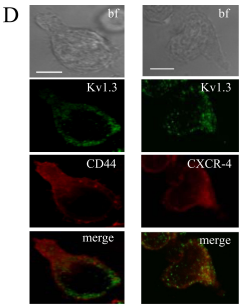
Flow Cytometry



Detection of CD44 by Flow Cytometry. MDA-MB-231 human breast cancer parental cell line WT and CD44 KO cells were labelled with a green or violet, fluorescent dye, respectively. WT and KO cells were mixed in a 1:1 ratio, fixed in 4% PFA and permeabilized in 0.1% saponin. 400,000 cells were stained with Mouse Anti-Human CD44 s Pan Specific Monoclonal Antibody (Catalog # BBA10) and a secondary antibody. Antibody staining was quantified, and representative images showing the staining intensity in the KO population (pink histogram, dashed line) compared to the WT cells (green histogram, solid line) are presented. Histograms with dotted lines represent secondary antibody-only controls in both WT and KO cells. Primary antibody concentration: 1 µg/mL. Image, protocol and testing courtesy of YCharOS Inc. (ycharos.com).

Immunocytochemistry/ Immunofluorescence



Detection of Human CD44 by Immunocytochemistry/Immunofluorescence Differential localization of Kv1.3 and KCa3.1 in migrating T cells.A. Distribution of Kv1.3 and KCa3.1 at the uropod. T cells were transiently transfected with either YFP-KCa3.1 or GFP-Kv1.3 (green) and stained with anti-CD44 antibody (uropod; red) without permeabilization. Yellow areas in the merge images indicate colocalization. Scale bar =5 µm. B. Distribution of Kv1.3 and KCa3.1 at the leading-edge. T cells, transfected with either YFP-KCa3.1 or GFP-Kv1.3 (green) and stained with anti-CXCR-4 antibody (leading-edge; red) without permeabilization, were analyzed by confocal microscopy. Colocalization between the two proteins is indicated by yellow areas in the merge images. Scale bar =5 µm. C. Correlation coefficients for KCa3.1 and Kv1.3 localization in the uropod (U) and leading-edge (L). The data are the average of n=15 cells for KCa3.1 at the U and n=8 at the L, and n=16 for Kv1.3 at the U and n=11 at the U from 2 healthy individuals. Statistical significance was established by one way ANOVA. D. Localization of native Kv1.3 in the leading-edge. T cells from one healthy individual were fixed and stained with extracellular anti-Kv1.3 antibody (green) together with antibodies either against CD44 (red; left) or CXCR-4 (red, right). Yellow colors in the merge images indicate strong correlation. Scale bar =5 µm. E. Average Correlation coefficients of native Kv1.3 with the leading-edge (L) (n=9) and the uropod (U) markers (n=11). Image collected and cropped by CiteAb from the following publication (https://dx.plos.org/10.1371/journal.pone.0043859), licensed under a CC-BY license. Not internally tested by R&D Systems.

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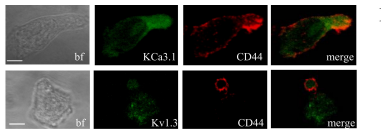

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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.5 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

CD44 is a ubiquitously expressed protein that is the major receptor for hyaluronan and exerts control over cell growth and migration (1-3). Human CD44 has a 20 amino acid (aa) signal sequence, an extracellular domain (ECD) with a 100 aa hyaluronan-binding disulfide-stabilized link region and a 325-530 aa stem region, a 21 aa transmembrane domain, and a 72 aa cytoplasmic domain. Within the stem, ten variably spliced exons (v1-10, exons 6-15) produce multiple protein isoforms (1-3). The standard or hematopoietic form, CD44s, does not include the variable segments (1-3). Cancer aggressiveness and T cell activation have been correlated with expression of specific isoforms (1, 3). With variable N- and O-glycosylation and splicing within the stalk, CD44 can range from 80-200 kDa (1). Within the N-terminal invariant portion of the ECD (aa 21-220), human CD44 shares 76%, 76%, 86%, 83%, and 79% identity with corresponding mouse, rat, equine, canine, and bovine CD44, respectively. The many reported functions of CD44 fall within three categories (1). First, CD44 binds hyaluronan and other ligands within the extracellular matrix and can function as a “platform” for growth factors and metalloproteinases. Second, CD44 can function as a co-receptor that modifies activity of receptors including MET and the ERBB family of tyrosine kinases. Third, the CD44 intracellular domain links the plasma membrane to the actin cytoskeleton via the ERM proteins, ezrin, radixin and moesin. CD44 can be synthesized in a soluble form (4) or may be cleaved at multiple sites by either membrane-type matrix metalloproteinases, or ADAM proteases to produce soluble ectodomains (5, 6). The cellular portion may then undergo gamma secretase-dependent intramembrane cleavage to form an Aβ-like transmembrane portion and a cytoplasmic signaling portion that affects gene expression (7, 8). These cleavage events are thought to promote metastasis by enhancing tumor cell motility and growth (1, 5).

References:

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