Mouse/Rat/Porcine/Equine CD44 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG

Catalog Number: AF6127

DESCRIPTION

Species Reactivity	Mouse/Rat/Porcine/Equine
Specificity	Detects mouse and rat CD44 in direct ELISAs and Western blots. In direct ELISAs, approximately 35% cross-reactivity with recombinant human CD44 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant mouse CD44 Gln25-Thr224 Accession # NP_033981
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

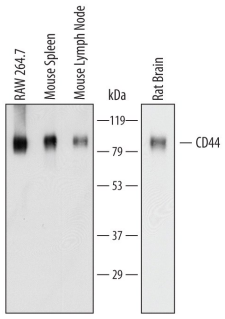
APPLICATIONS

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	1 µg/mL	See Below
Flow Cytometry	2.5 µg/10^6 cells	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Simple Western	10 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.	
Blockade of Receptor-ligand Interaction	In a functional ELISA, 1-6 µg/mL of this antibody will block 50% of the binding of 25 ng/mL biotinylated Hyaluronan (132 kDa) to immobilized Recombinant Mouse CD44 Fc Chimera (Catalog # 6127-CD) coated at 1 µg/mL (100 µL/well). At 20 µg/mL, this antibody will block >90% of the binding.	

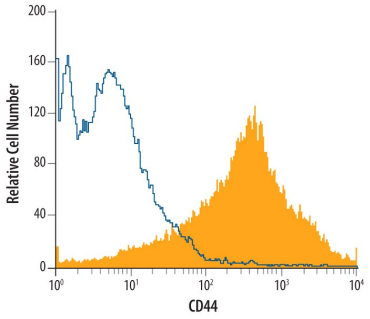
DATA

Western Blot



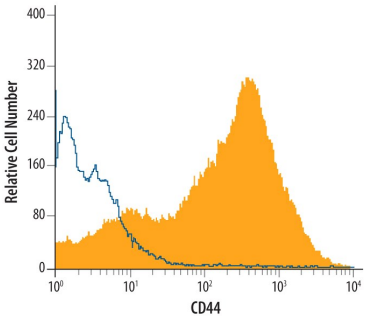
Detection of Mouse and Rat CD44 by Western Blot. Western blot shows lysates of RAW 264.7 mouse monocyte/macrophage cell line, mouse spleen tissue, mouse lymph node tissue, and rat brain tissue. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for CD44 at approximately 80 to 100 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Flow Cytometry



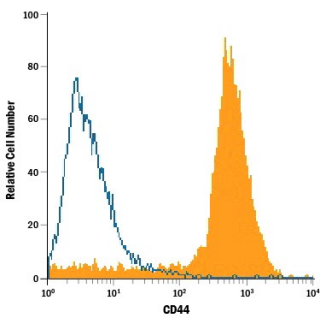
Detection of CD44 in Mouse Splenocytes by Flow Cytometry. Mouse splenocytes were stained with Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127, filled histogram) or control antibody (Catalog # 5-001-A, open histogram), followed by Allophycocyanin-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # F0127).

Flow Cytometry



Detection of CD44 in Rat Splenocytes by Flow Cytometry. Rat splenocytes were stained with Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127, filled histogram) or control antibody (Catalog # 5-001-A, open histogram), followed by Allophycocyanin-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # F0127).

Flow Cytometry



Detection of CD44 in Porcine Mesenchymal Stem Cells by Flow Cytometry. Porcine mesenchymal stem cells were stained with Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127, filled histogram) or isotype control antibody (Catalog # 5-001-A, open histogram), followed by Phycoerythrin-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # F0126).

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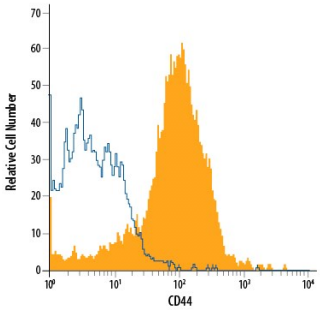
Antigen Affinity-purified Polyclonal Sheep IgG

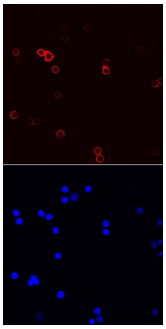
Catalog Number: AF6127

Flow Cytometry



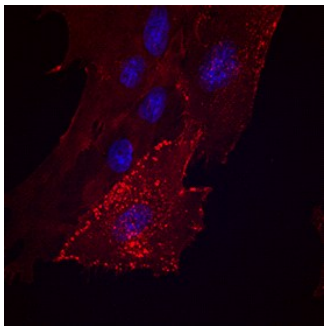
Detection of CD44 in Equine PBMCs by Flow Cytometry. Equine peripheral blood mononuclear cells (PBMCs) were stained with Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127, filled histogram) or isotype control antibody (Catalog # 5-001-A, open histogram), followed by Phycoerythrin-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # F0126).

Immunocytochemistry



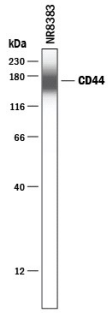
CD44 in Mouse Splenocytes. CD44 was detected in immersion fixed mouse splenocytes using Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red, upper panel; Catalog # NL010) and counterstained with DAPI (blue, lower panel). Specific staining was localized to cell surfaces. View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunocytochemistry



CD44 in Porcine Mesenchymal Stem Cells. CD44 was detected in immersion fixed porcine mesenchymal stem cells using Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces. View our protocol for Fluorescent ICC Staining of Stem Cells on Coverslips.

Simple Western



Detection of Rat CD44 by Simple Western™. Simple Western lane view shows lysates of NR8383 rat alveolar macrophage cell line, loaded at 0.2 mg/mL. A specific band was detected for CD44 at approximately 169 kDa (as indicated) using 10 µg/mL of Sheep Anti-Mouse/Rat/Porcine/Equine CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6127) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
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.

BACKGROUND

CD44 is a ubiquitously expressed protein that is the major receptor for hyaluronan and exerts control over cell growth and migration (1 - 5). Mouse CD44 has a 22 amino acid (aa) signal sequence, an extracellular domain (ECD) with a 100 aa hyaluronan-binding disulfide-stabilized link region and a 48-463 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 (1-5). The standard or hematopoietic form, CD44H, does not include the variable segments (1-5). Cancer aggressiveness and T cell activation have been correlated with expression of specific isoforms (2, 4). With variable N- and O-glycosylation and splicing within the stalk, CD44 can range from 80 to 200 kDa (1, 2). Within the N-terminal invariant portion of the ECD (aa 23-222), mouse CD44 shares 92%, 77%, 77%, 79% and 71% identity with corresponding rat, human, equine, canine and bovine CD44, respectively. The many reported functions of CD44 fall within three categories (1, 2). First, CD44 binds hyaluronan and other ligands within the extracellular matrix and can function as a "platform" for growth factors and metalloproteinases. Second, CD44 is 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 (6, 7). 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 (8, 9). These cleavage events are thought to promote metastasis by enhancing tumor cell motility and growth (1, 2, 6).

References:

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