

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CD44 in direct ELISAs and Western blots. In direct ELISAs, approximately 25% cross-reactivity with recombinant mouse CD44 is observed and approximately 12% cross-reactivity with recombinant rat CD44 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human CD44 Gln21-Pro220 Accession # P16070
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.

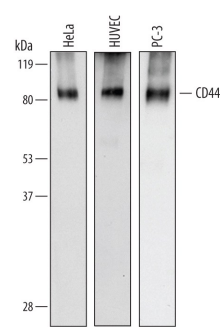
APPLICATIONS

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	0.2 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	10 µg/mL	See Below

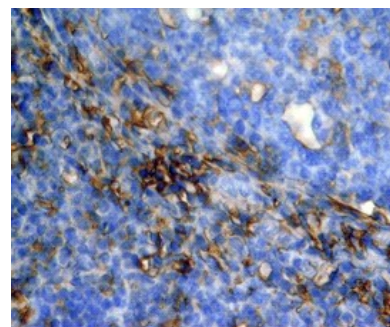
DATA

Western Blot



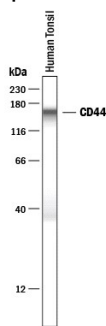
Detection of Human CD44 by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line, HUVEC human umbilical vein endothelial cells, and PC-3 human prostate cancer cell line. PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3660) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). Specific bands were detected for CD44 at approximately 85 kDa (as indicated). This experiment was conducted under reducing conditions and using *Immunoblot Buffer Group 1*.

Immunohistochemistry



CD44 in Human Tonsil. CD44 was detected in immersion fixed paraffin-embedded sections of human tonsil using Sheep Anti-Human CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3660) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to cell surfaces and cytoplasm. View our protocol for *Chromogenic IHC Staining of Paraffin-embedded Tissue Sections*.

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 153 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human CD44 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3660) 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. <ul style="list-style-type: none"> ● 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 (1-3). 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, CD44H, 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 to 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 A β -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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2. Sreaton, G.R. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**:12160.
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6. Nakamura, H. *et al.* (2004) *Cancer Res.* **64**:876.
7. Murakami, D. *et al.* (2003) *Oncogene* **22**:1511.
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