

#3578 Store at -20°C

CD44 Antibody



100 µl
 (10 Western mini-blot)

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New 08/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H	80 kDa	Rabbit

Background: CD44 is a type I transmembrane glycoprotein that mediates cell-cell and cell-matrix interaction through its affinity for hyaluronic acid (HA) and possibly through other parts of the extracellular matrix (ECM). CD44 is highly polymorphic, possesses a number of alternative splice variants and undergoes extensive post-translational modifications (1-2). Increased surface levels of CD44 are characteristic of T cell activation, and expression of the protein is upregulated during the inflammatory response. Interactions between CD44 and HER2 have been linked to an increase in ovarian carcinoma cell growth (1-3).

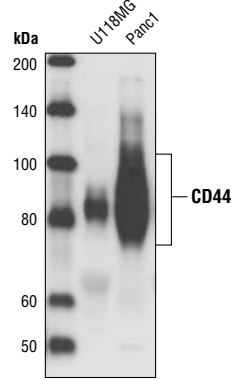
CD44 interacts with ezrin, radixin and moesin (ERM), linking the actin cytoskeleton to the plasma membrane and the ECM (4-6). CD44 is constitutively phosphorylated at Ser325 in resting cells. Activation of PKC results in phosphorylation of Ser291, dephosphorylation of Ser325, disassociation of ezrin from CD44, and directional motility (4).

Specificity/Sensitivity: CD44 Antibody detects endogenous levels of total CD44 protein.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) surrounding Leu135 of human CD44. Antibodies are purified by peptide affinity chromatography.

Background References:

- (1) Goodison, S. et al. (1999) *Mol. Pathol.* 52, 189–196.
- (2) Cichy, J. and Puré, E. (2003) *J. Cell Biol.* 161, 839–843.
- (3) Bourguignon, L.Y. et al. (1997) *J. Biol. Chem.* 272, 27913–27918.
- (4) Legg, J.W. et al. (2002) *Nat. Cell Biol.* 4, 399–407.
- (5) Yonemura, S. et al. (1998) *J. Cell Biol.* 140, 885–895.
- (6) Tsukita, S. et al. (1994) *J. Cell Biol.* 126, 391–401.



Western blot analysis of extracts from U118MG and Panc1 cells, using CD44 Antibody.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.

Recommended Antibody Dilutions:
 Western blotting 1:1000

- Companion Products:**
- CD3e (CD3-12) Rat mAb #4443
 - CD4 (Edu-2) Mouse mAb #3563
 - CD8 (RIV11) Mouse mAb #3572
 - CD10 (CB-CALLA) Mouse mAb #3565
 - CD13 (B-F10) Mouse mAb #3566
 - CD14 (5A3B11B5) Mouse mAb #3573
 - CD16 (FcγIII) (CB-16) Mouse mAb #3567
 - CD34 (IC0115) Mouse mAb #3569
 - CD44 (156-3C11) Mouse mAb #3570
 - CD56 (NCAM) (123C3) Mouse mAb #3576
 - Phospho-Ezrin (Thr567)/Radixin (Thr564)/Moesin (Thr558) Antibody #3141
 - Ezrin/Radixin/Moesin Antibody #3142
 - Phospho-PKC Antibody Sampler Kit #9921
 - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder #7727
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
 F—Flow cytometry E—ELISA D—DELFIA®
 Z—zebra fish B—bovine All—all species expected

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.