

## Anti-CD44 Antibody Picoband™

Catalog Number: A00052-2

### About CD44

CD44 is also known as LHR or MC56. The protein encoded by this gene is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. It is a receptor for hyaluronic acid (HA) and can also interact with other ligands, such as osteopontin, collagens, and matrix metalloproteinases (MMPs). This protein participates in a wide variety of cellular functions including lymphocyte activation, recirculation and homing, hematopoiesis, and tumor metastasis. Transcripts for this gene undergo complex alternative splicing that results in many functionally distinct isoforms, however, the full length nature of some of these variants has not been determined. Alternative splicing is the basis for the structural and functional diversity of this protein, and may be related to tumor metastasis.

### Overview

Product Name	Anti-CD44 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-CD44 Antibody Picoband™ catalog # A00052-2. Tested in WB, IHC, ICC/IF, FCM, ELISA applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
Storage Instructions	At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freezing and thawing.
Host	Rabbit
Uniprot ID	P16070

### Technical Details

Immunogen	E.coli-derived human CD44 recombinant protein (Position: Q21-E518). Human CD44 shares 66.5% amino acid (aa) sequence identity with mouse CD44.
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this

kit.

If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

Some PubMed article(s) citing the expression level of this target are as follows:

Boster Bio's internal QC testing used:

Western blot, 0.25-0.5 µg/ml, Human

Immunohistochemistry, 2-5 µg/ml, Human, Mouse, Rat

Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human

Flow Cytometry (Fixed), 1-3 µg/1x10<sup>6</sup> cells, Human

ELISA, 0.1-0.5 µg/ml, Human

## Anti-CD44 Antibody Picoband™ (A00052-2) Images

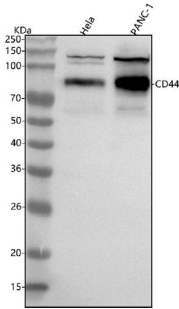


Figure 1. Western blot analysis of CD44 using anti-CD44 antibody (A00052-2).

Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,  
Lane 2: human PANC-1 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-CD44 antigen affinity purified polyclonal antibody (Catalog # A00052-2) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for CD44 at approximately 85 kDa. The expected band size for CD44 is at 82 kDa.

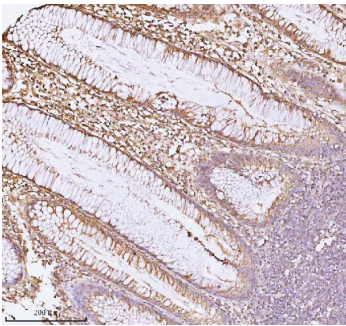


Figure 2. IHC analysis of CD44 using anti-CD44 antibody (A00052-2).

CD44 was detected in a paraffin-embedded section of human colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD44 Antibody (A00052-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

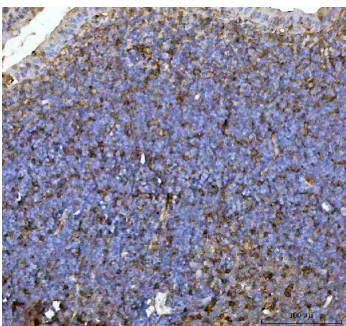
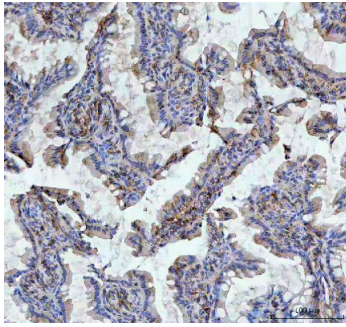


Figure 3. IHC analysis of CD44 using anti-CD44 antibody (A00052-2).

CD44 was detected in a paraffin-embedded section of mouse lymphaden tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD44 Antibody (A00052-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

Figure 4. IHC analysis of CD44 using anti-CD44 antibody (A00052-2).



CD44 was detected in a paraffin-embedded section of rat colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD44 Antibody (A00052-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

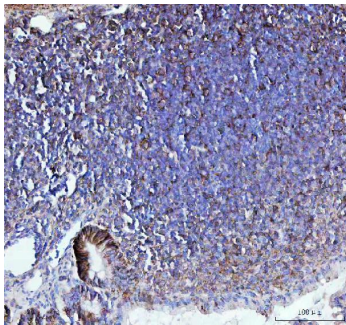


Figure 5. IHC analysis of CD44 using anti-CD44 antibody (A00052-2).

CD44 was detected in a paraffin-embedded section of rat colon tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-CD44 Antibody (A00052-2) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

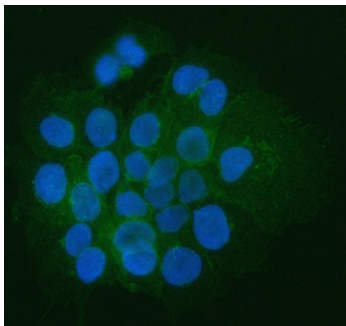


Figure 6. IF analysis of CD44 using anti-CD44 antibody (A00052-2).

CD44 was detected in an immunocytochemical section of A431 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-CD44 Antibody (A00052-2) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

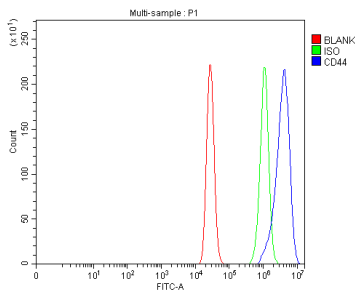


Figure 7. Flow Cytometry analysis of MCF-7 cells using anti-CD44 antibody (A00052-2).

Overlay histogram showing MCF-7 cells stained with A00052-2 (Blue line). The cells were fixed with 4% paraformaldehyde and blocked with 10% normal goat serum. And then incubated with rabbit anti-CD44 Antibody (A00052-2, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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