

Anti-CAPN5 Antibody Picoband™

Catalog Number: A08350-1

About CAPN5

Calpain-5 is a protein that in humans is encoded by the CAPN5 gene. Calpains are calcium-dependent cysteine proteases involved in signal transduction in a variety of cellular processes. A functional calpain protein consists of an invariant small subunit and 1 of a family of large subunits. CAPN5 is one of the large subunits. Unlike some of the calpains, CAPN5 and CAPN6 lack a calmodulin-like domain IV. Because of the significant similarity to *Caenorhabditis elegans* sex determination gene tra-3, CAPN5 is also called as HTRA3.

Overview

Product Name	Anti-CAPN5 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-CAPN5 Antibody Picoband™ catalog # A08350-1. Tested in WB, ICC/IF, FCM, ELISA applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, Flow Cytometry, IF, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl, 0.2 mg Na ₂ HPO ₄ .
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	O15484

Technical Details

Immunogen	E.coli-derived human CAPN5 recombinant protein (Position: N583-N616). Human CAPN5 shares 84.8% and 78.8% amino acid (aa) sequence identity with mouse and rat CAPN5, respectively.
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 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, 0.1-0.25 µg/ml, Human, Mouse, Rat

Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human

Flow Cytometry (Fixed), 1-3 µg/1x10⁶ cells, Human

ELISA, 0.1-0.5 µg/ml, Human

Anti-CAPN5 Antibody Picoband™ (A08350-1) Images

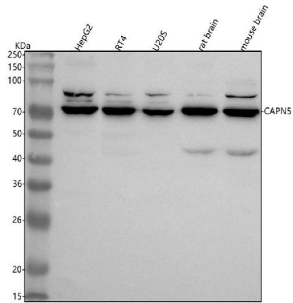


Figure 1. Western blot analysis of CAPN5 using anti-CAPN5 antibody (A08350-1).

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 HepG2 whole cell lysates,

Lane 2: human RT4 whole cell lysates,

Lane 3: human U2OS whole cell lysates,

Lane 4: rat brain tissue lysates,

Lane 5: mouse brain tissue 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-CAPN5 antigen affinity purified polyclonal antibody (Catalog # A08350-1) at 0.25 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 CAPN5 at approximately 73 kDa. The expected band size for CAPN5 is at 73 kDa.

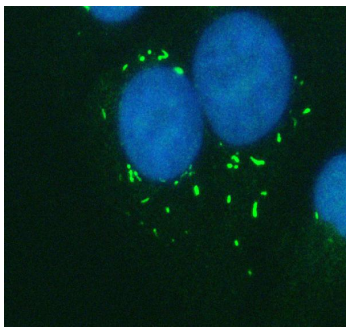


Figure 2. IF analysis of CAPN5 using anti-CAPN5 antibody (A08350-1).

CAPN5 was detected in an immunocytochemical section of U2OS 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-CAPN5 Antibody (A08350-1) overnight at 4°C. DyLight®550 Conjugated Goat Anti-Rabbit IgG (BA1135) 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.

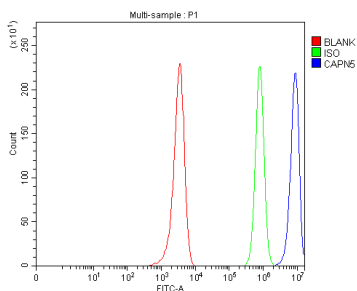


Figure 3. Flow Cytometry analysis of U251 cells using anti-CAPN5 antibody (A08350-1).

Overlay histogram showing U251 cells stained with A08350-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-CAPN5 Antibody (A08350-1, 1 ug/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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