

## Anti-Calpastatin/CAST Antibody Picoband™

Catalog Number: A00337

### About CAST

Calpastatin is a protein that in humans is encoded by the CAST gene. It is mapped to 5q15. The protein encoded by this gene is an endogenous calpain (calcium-dependent cysteine protease) inhibitor. It consists of an N-terminal domain L and four repetitive calpain-inhibition domains (domains 1-4), and it is involved in the proteolysis of amyloid precursor protein. The calpain/calpastatin system is involved in numerous membrane fusion events, such as neural vesicle exocytosis and platelet and red-cell aggregation. The encoded protein is also thought to affect the expression levels of genes encoding structural or regulatory proteins. Alternatively spliced transcript variants encoding different isoforms have been described.

### Overview

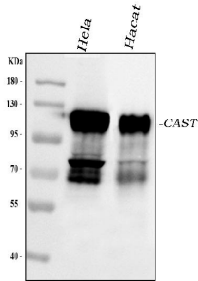
Product Name	Anti-Calpastatin/CAST Antibody Picoband™
Reactive Species	Human
Description	Boster Bio Anti-Calpastatin/CAST Antibody Picoband™ catalog # A00337. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na <sub>2</sub> HPO <sub>4</sub> , 0.05mg NaN <sub>3</sub> .
Storage Instructions	Store 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 freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P20810

### Technical Details

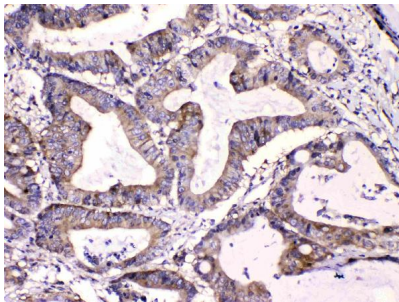
Immunogen	A synthetic peptide corresponding to a sequence in the middle region of human Calpastatin, different from the related mouse sequence by fifteen amino acids, and from the related rat sequence by eleven amino acids.
Predicted Reactive Species	Chicken
Recommended Detection Systems	Boster recommends 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.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml, Human</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat</p> <p>Immunocytochemistry/Immunofluorescence, 2ug/ml, Human</p> <p>Flow Cytometry, 1-3ug/1x10<sup>6</sup> cells, Human</p>

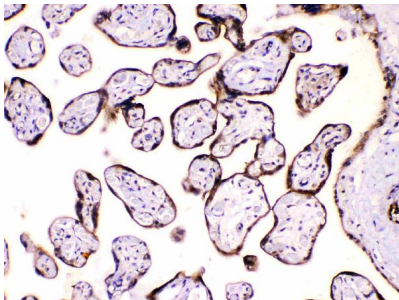
## Anti-Calpastatin/CAST Antibody Picoband™ (A00337) Images



**Figure 1. Western blot analysis of Calpastatin using anti-Calpastatin antibody (A00337).**  
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 Hacat 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-Calpastatin antigen affinity purified polyclonal antibody (Catalog # A00337) 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 Calpastatin at approximately 120 kDa. The expected band size for Calpastatin is at 77 kDa.

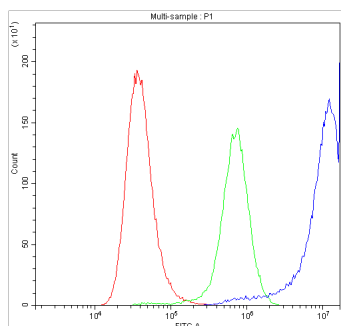


**Figure 2. IHC analysis of Calpastatin using anti-Calpastatin antibody (A00337).**  
Calpastatin was detected in paraffin-embedded section of human intestinal cancer tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Calpastatin Antibody (A00337) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.



**Figure 3. IHC analysis of Calpastatin using anti-Calpastatin antibody (A00337).**  
Calpastatin was detected in paraffin-embedded section of human placenta tissues. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml rabbit anti-Calpastatin Antibody (A00337) overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC)(Catalog # SA1022) with DAB as the chromogen.

**Figure 4. Flow Cytometry analysis of A549 cells using anti-**



Calpastatin antibody (A00337).  
Overlay histogram showing A549 cells stained with A00337 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Calpastatin Antibody (A00337, 1 $\mu$ g/1 $\times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 $\mu$ g/1 $\times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu$ g/1 $\times 10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

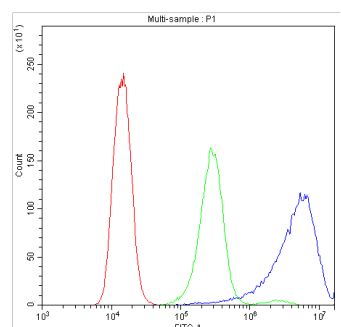


Figure 5. Flow Cytometry analysis of A431 cells using anti-Calpastatin antibody (A00337).  
Overlay histogram showing A431 cells stained with A00337 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Calpastatin Antibody (A00337, 1 $\mu$ g/1 $\times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 $\mu$ g/1 $\times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu$ g/1 $\times 10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

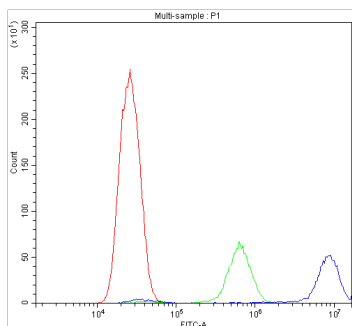


Figure 6. Flow Cytometry analysis of U20S cells using anti-Calpastatin antibody (A00337).  
Overlay histogram showing U20S cells stained with A00337 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Calpastatin Antibody (A00337, 1 $\mu$ g/1 $\times 10^6$  cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 $\mu$ g/1 $\times 10^6$  cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu$ g/1 $\times 10^6$ ) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

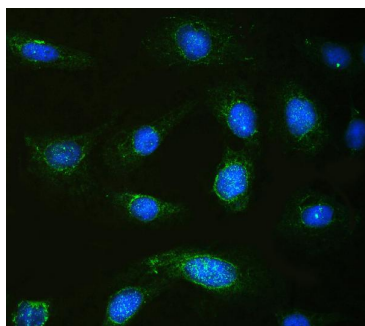


Figure 7. IF analysis of Calpastatin using anti-Calpastatin antibody (A00337).  
Calpastatin was detected in immunocytochemical section of U20S cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 $\mu$ g/mL rabbit anti-Calpastatin Antibody (A00337) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) was used as secondary antibody at 1:100 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.

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