

Anti-Calpain 2 CAPN2 Antibody Picoband™ (monoclonal, 8I6)

Catalog # ABO14821

Product Information

Application	WB, IHC, IF, ICC, FC
Primary Accession	P17655
Host	Mouse
Isotype	Mouse IgG2a
Reactivity	Human, Mouse, Monkey
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Calpain 2 CAPN2 Antibody Picoband™ (monoclonal, 8I6) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Monkey, Mouse.

Additional Information

Gene ID	824
Other Names	Calpain-2 catalytic subunit, 3.4.22.53, Calcium-activated neutral proteinase 2, CANP 2, Calpain M-type, Calpain large polypeptide L2, Calpain-2 large subunit, Millimolar-calpain, M-calpain, CAPN2, CANPL2
Calculated MW	79995
Application Details	Western blot, 0.1-0.5 µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml Immunocytochemistry/Immunofluorescence, 2 µg/ml Flow Cytometry, 1-3 µg/1x10 ⁶ cells
Subcellular Localization	Cytoplasm. Cell membrane. Translocates to the plasma membrane upon Ca (2+) binding.
Tissue Specificity	Ubiquitous.
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names	Clone: 8I6
Immunogen	E. coli-derived human Calpain 2 recombinant protein (Position: R500-L700). Human Calpain 2 shares 93.5% and 92.5% amino acid (aa) sequence identity with mouse and rat Calpain 2, respectively.

Cross Reactivity

No cross-reactivity with other proteins.

Storage

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.

Protein Information**Name**

CAPN2

Synonyms

CANPL2

Function

Calcium-regulated non-lysosomal thiol-protease which catalyzes limited proteolysis of substrates involved in cytoskeletal remodeling and signal transduction. Proteolytically cleaves MYOC at 'Arg-226' (PubMed:[17650508](#)). Proteolytically cleaves CPEB3 following neuronal stimulation which abolishes CPEB3 translational repressor activity, leading to translation of CPEB3 target mRNAs (By similarity).

Cellular Location

Cytoplasm. Cell membrane. Note=Translocates to the plasma membrane upon Ca(2+) binding

Tissue Location

Ubiquitous.

Background

Calpain-2 catalytic subunit is a protein that in humans is encoded by the CAPN2 gene. The calpains, calcium-activated neutral proteases, are nonlysosomal, intracellular cysteine proteases. The mammalian calpains include ubiquitous, stomach-specific, and muscle-specific proteins. The ubiquitous enzymes consist of heterodimers with distinct large, catalytic subunits associated with a common small, regulatory subunit. This gene encodes the large subunit of the ubiquitous enzyme, calpain 2. Multiple heterogeneous transcriptional start sites in the 5' UTR have been reported.

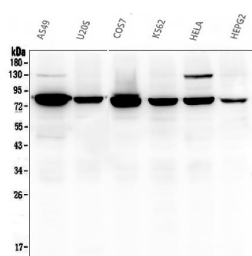
Images

Figure 1. Western blot analysis of Calpain 2 using anti-Calpain 2 antibody (M03492).

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 50ug of sample under reducing conditions.

Lane 1: A549 whole cell lysates,

Lane 2: U20S whole cell lysates,

Lane 3: COS-7 whole cell lysates,

Lane 4: K562 whole cell lysates,

Lane 5: HELA whole cell lysates,

Lane 6: HEPG2 whole cell lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-Calpain 2 antigen affinity purified monoclonal antibody (Catalog # M03492) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse 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 # EK1001) with Tanon 5200 system. A specific band was detected for Calpain 2 at approximately 80KD. The expected band size for Calpain 2 is at 80KD.

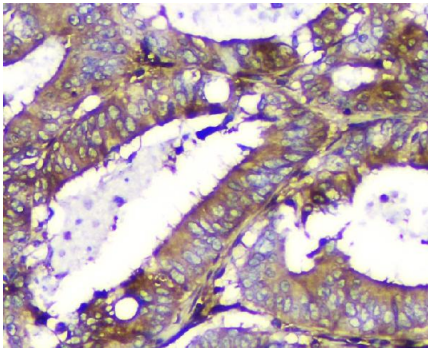


Figure 2. IHC analysis of Calpain 2 using anti-Calpain 2 antibody (M03492).

Calpain 2 was detected in paraffin-embedded section of human intestinal cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Calpain 2 Antibody (M03492) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

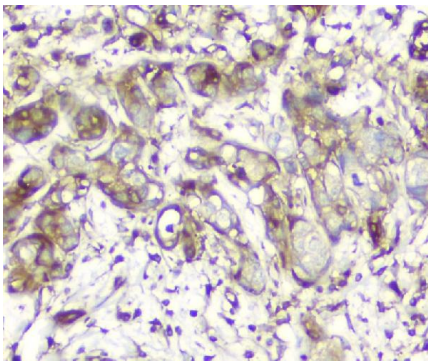


Figure 3. IHC analysis of Calpain 2 using anti-Calpain 2 antibody (M03492).

Calpain 2 was detected in paraffin-embedded section of human mammary cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Calpain 2 Antibody (M03492) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

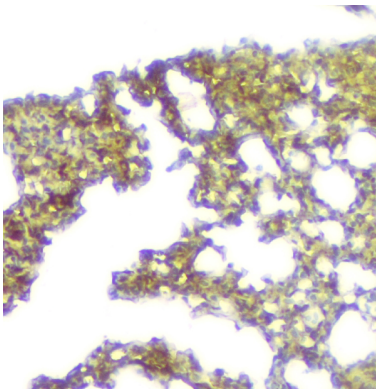


Figure 4. IHC analysis of Calpain 2 using anti-Calpain 2 antibody (M03492).

Calpain 2 was detected in paraffin-embedded section of mouse lung tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 µg/ml mouse anti-Calpain 2 Antibody (M03492) overnight at 4°C. Biotinylated goat anti-mouse 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 # SA1021) with DAB as the chromogen.

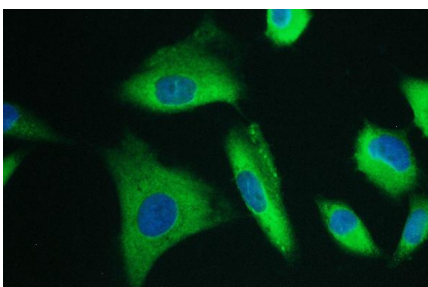


Figure 5. IF analysis of Calpain 2 using anti-Calpain 2 antibody (M03492).

Calpain 2 was detected in immunocytochemical section of A549 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 µg/mL mouse anti-Calpain 2 Antibody (M03492) overnight at 4°C. DyLight®488 Conjugated Goat Anti-mouse IgG (BA1126) 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.

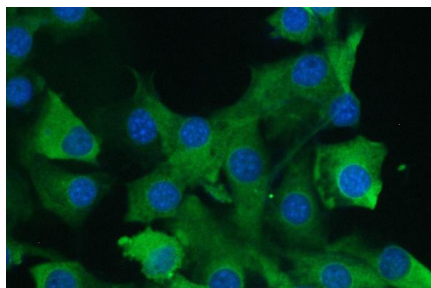


Figure 6. IF analysis of Calpain 2 using anti-Calpain 2 antibody (M03492). Calpain 2 was detected in immunocytochemical section of U251 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2 $\mu\text{g}/\text{mL}$ mouse anti-Calpain 2 Antibody (M03492) overnight at 4°C. DyLight®488 Conjugated Goat Anti-mouse IgG (BA1126) 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.

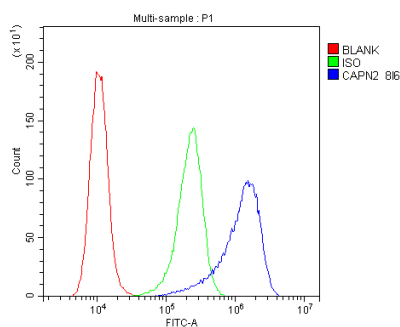


Figure 7. Flow Cytometry analysis of A431 cells using anti-Calpain 2 antibody (M03492). Overlay histogram showing A431 cells stained with M03492 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Calpain 2 Antibody (M03492, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

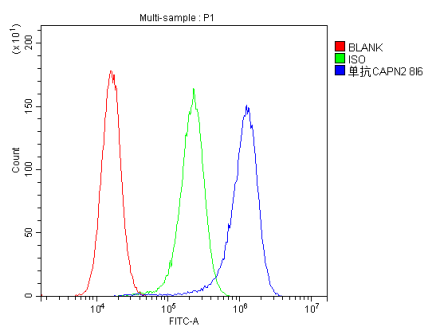


Figure 8. Flow Cytometry analysis of U87 cells using anti-Calpain 2 antibody (M03492). Overlay histogram showing U87 cells stained with M03492 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Calpain 2 Antibody (M03492, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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