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antibody and ELISA experts

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Anti-Cystatin C/CST3 Antibody Picoband™ (monoclonal, 4H8)

Catalog Number: M00961-1

About CST3

Cystatin C or cystatin 3, a protein encoded by the CST3 gene, is mainly used as a biomarker of kidney function. Recently, it has been studied for its role in predicting new-onset or deteriorating cardiovascular disease. It also seems to play a role in brain disorders involving amyloid, such as Alzheimer's disease. In humans, all cells with a nucleus (cell core containing the DNA) produce cystatin C as a chain of 120 amino acids. It is found in virtually all tissues and body fluids. It is a potent inhibitor of lysosomal proteinases (enzymes from a special subunit of the cell that break down proteins) and probably one of the most important extracellular inhibitors of cysteine proteases (it prevents the breakdown of proteins outside the cell by a specific type of protein degrading enzymes). Cystatin C belongs to the type 2 cystatin gene family.

Overview

Product Name	Anti-Cystatin C/CST3 Antibody Picoband™ (monoclonal, 4H8)
Reactive Species	Human
Description	Boster Bio Anti-Cystatin C/CST3 Antibody Picoband™ (monoclonal, 4H8) catalog # M00961-1. Tested in ELISA, Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.
Application	ELISA, Flow Cytometry, IF, IHC, ICC, WB
Clonality	Monoclonal 4H8
Formulation	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg NaN3.
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	Mouse
Uniprot ID	P01034

Technical Details

Immunogen	E. coli-derived human Cystatin C recombinant protein (Position: K31-A146).
Predicted Reactive Species	Hepatitis Virus
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Mouse IgG (EK1001) for Western blot, and HRP Conjugated anti-Mouse IgG Super Vision Assay Kit (SV0001-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Mouse IgG2a
Form	Lyophilized

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Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/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, 0.1-0.5ug/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml Immunocytochemistry/Immunofluorescence, 2ug/ml Flow Cytometry, 1-3ug/1x10^6 cells ELISA (Cap), 1-5ug/ml

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Anti-Cystatin C/CST3 Antibody Picoband™ (monoclonal, 4H8) (M00961-1) Images

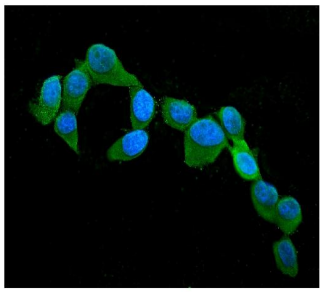

Figure 1. IF analysis of CST3 using anti-CST3 antibody (M00961-1).
CST3 was detected in immunocytochemical section of MCF7 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 2ug/mL mouse anti-CST3 Antibody (M00961-1) 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.

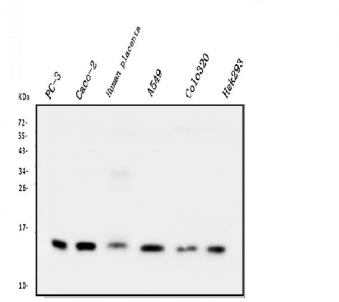

Figure 2. Western blot analysis of Cystatin C/CST3 using anti-Cystatin C/CST3 antibody (M00961-1).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human PC-3 whole cell lysates,
Lane 2: human CACO-2 whole cell lysates,
Lane 3: human placenta tissue lysates,
Lane 4: human A549 whole cell lysates,
Lane 5: human COLO320 whole cell lysates,
Lane 6: human HEK293 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-Cystatin C/CST3 antigen affinity purified monoclonal antibody (Catalog # M00961-1) 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-mouse IgG-HRP secondary antibody at a dilution of 1:10000 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 Cystatin C/CST3 at approximately 15KD. The expected band size for Cystatin C/CST3 is at 15KD.

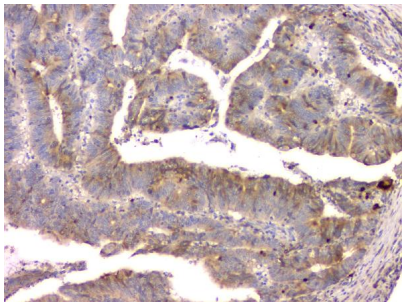

Figure 3. IHC analysis of Cystatin C/CST3 using anti-Cystatin C/CST3 antibody (M00961-1).
Cystatin C/CST3 was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-Cystatin C/CST3 Antibody (M00961-1) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the

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chromogen.

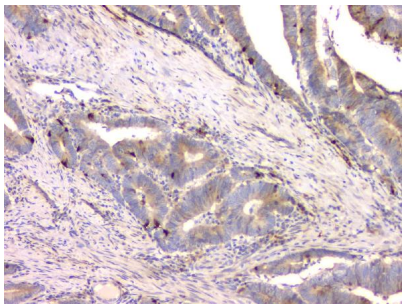

Figure 4. IHC analysis of Cystatin C/CST3 using anti-Cystatin C/CST3 antibody (M00961-1).
Cystatin C/CST3 was detected in paraffin-embedded section of human colon cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-Cystatin C/CST3 Antibody (M00961-1) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

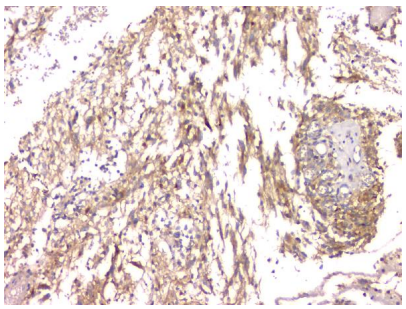

Figure 5. IHC analysis of Cystatin C/CST3 using anti-Cystatin C/CST3 antibody (M00961-1).
Cystatin C/CST3 was detected in paraffin-embedded section of human glioma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1ug/ml mouse anti-Cystatin C/CST3 Antibody (M00961-1) 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 Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

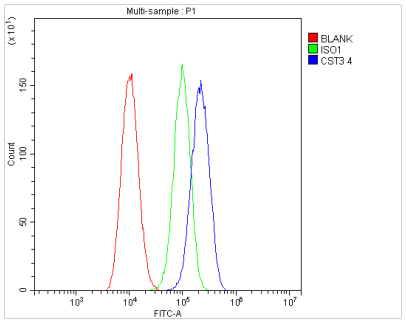

Figure 6. Flow Cytometry analysis of A549 cells using anti-Cystatin C/CST3 antibody (M00961-1).
Overlay histogram showing A549 cells stained with M00961-1 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Cystatin C/CST3 Antibody (M00961-1, 1ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

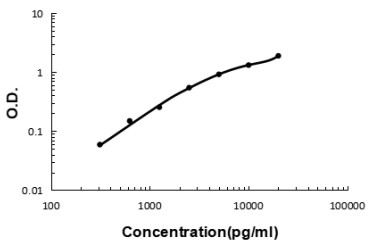

Figure 7. Sandwich ELISA - Recombinant human Cystatin C/CST3 protein standard curve.
Use in combination with reagents from Human Cystatin C/CST3 ELISA Kit EZ-Set (DIY Antibody Pairs) (EZ0678).

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