

Anti-Stefin B CSTB Antibody Picoband™ (monoclonal, 2B6)

Catalog # ABO14337

Product Information

Application	WB, IHC, IHC-F, ICC, FC
Primary Accession	P04080
Host	Mouse
Isotype	Mouse IgG1
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-Stefin B CSTB Antibody Picoband™ (monoclonal, 2B6) . Tested in Flow Cytometry, IHC, IHC-F, ICC, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	1476
Other Names	Cystatin-B, CPI-B, Liver thiol proteinase inhibitor, Stefin-B, CSTB, CST6, STFB
Calculated MW	11140
Application Details	Western blot, 0.1-0.5 µg/ml Immunohistochemistry (Paraffin-embedded Section), 0.5-1 µg/ml Immunohistochemistry (Frozen Section), 0.5-1 µg/ml Immunocytochemistry, 0.5-1 µg/ml Flow Cytometry, 1-3 µg/1x10 ⁶ cells
Subcellular Localization	Cytoplasm
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na ₂ HPO ₄ , 0.05mg NaN ₃ .
Clone Names Immunogen	Clone: 2B6 E. coli-derived human Stefin B recombinant protein (Position: M1-F98). Human Stefin B shares 78.6 % amino acid (aa) sequence identity with both mouse and rat Stefin B.
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	CSTB
Synonyms	CST6, STFB
Function	This is an intracellular thiol proteinase inhibitor. Tightly binding reversible inhibitor of cathepsins L, H and B.
Cellular Location	Cytoplasm. Nucleus

Background

Cystatin B (CSTB), also called STFB, is a small protein that is a member of the superfamily of cysteine protease inhibitors. It has been isolated from human spleen and liver and its amino acid sequence has been fully determined. The cystatin B gene is located on 21q22.3. It is widely distributed and is localized mostly intracellularly, but has been found extracellularly. The protein is able to form a dimer stabilized by noncovalent forces, inhibiting papain and cathepsins L, H and B. Its role is thought to be as a protector against the proteinases leaking from lysosomes. A cystatin B multiprotein complex might have a specific cerebellar function, and that the loss of this function might contribute to the etiopathogenesis of EPM1. Upon differentiation to myotubes, CSTB becomes excluded from the nucleus and lysosomes, suggesting that the subcellular distribution of CSTB is dependent on the differentiation status of the cell.

Images

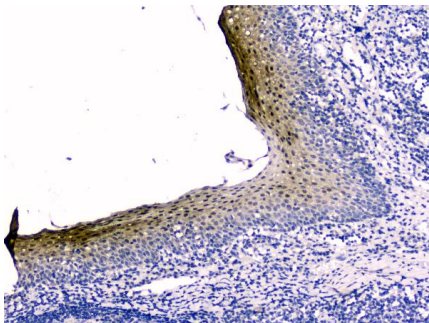


Figure 2. IHC analysis of Stefin B using anti-Stefin B antibody (M02794).

Stefin B was detected in paraffin-embedded section of human tonsil tissue. 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 2 µg/ml mouse anti-Stefin B Antibody (M02794) 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 1. Western blot analysis of Stefin B using anti-Stefin B antibody (M02794).

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 U-87MG whole cell lysates,
Lane 2: human SW620 whole cell lysates,
Lane 3: human U-937 whole cell lysates,
Lane 4: human Caco-2 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-Stefin B antigen affinity purified monoclonal

antibody (Catalog # M02794) 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 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system.

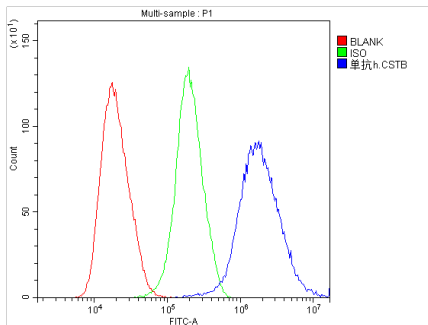


Figure 3. Flow Cytometry analysis of PC-3 cells using anti-Stefin B antibody (M02794).

Overlay histogram showing PC-3 cells stained with M02794 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-Stefin B Antibody (M02794, 1 µg/1x10⁶ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10⁶ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10⁶) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

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