Anti-Cystatin-B Antibody [JE63-34]

HA720084

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Rat
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 11 kDa
Clone number:	JE63-34

Description: The cystatin superfamily encompasses proteins that contain multiple cystatin-like sequences. Some of the members are active cysteine protease inhibitors, while others have lost or perhaps never acquired this inhibitory activity. There are three inhibitory families in the superfamily, including the type 1 cystatins (stefins), type 2 cystatins and kininogens. This gene encodes a stefin that functions as an intracellular thiol protease inhibitor. The protein is able to form a dimer stabilized by noncovalent forces, inhibiting papain and cathepsins l, h and b. The protein is thought to play a role in protecting against the proteases leaking from lysosomes. Evidence indicates that mutations in this gene are responsible for the primary defects in patients with progressive myoclonic epilepsy (EPM1). One type of mutation responsible for EPM1 is the expansion in the promoter region of this gene of a CCCCGCCCCGCG repeat from 2-3 copies to 30-78 copies.

Immunogen: Synthetic peptide within human Cystatin-B aa 49-98/98.

Positive control: PC-12 cell lysates, rat bladder tissue, rat tongue tissue, human esophagus tissue, PC-3M.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P04080 Human | P01041 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
FC	1:500-1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

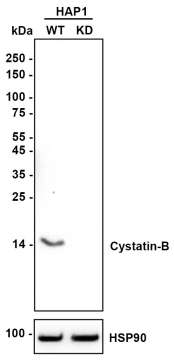

Fig1: Western blot analysis of Cystatin-B on different lysates with Rabbit anti-Cystatin-B antibody (HA720084) at 1/1,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Cystatin-B KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 11 kDa
Observed band size: 15 kDa

Exposure time: 180 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA720084) at 1/1,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

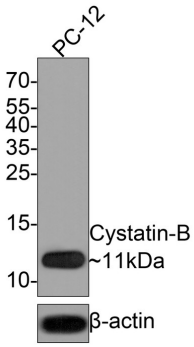

Fig2: Western blot analysis of Cystatin-B on PC-12 cell lysates with Rabbit anti-Cystatin-B antibody (HA720084) at 1/1,000 dilution.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 11 kDa
Observed band size: 11 kDa

Exposure time: 30 seconds;

15% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA720084) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

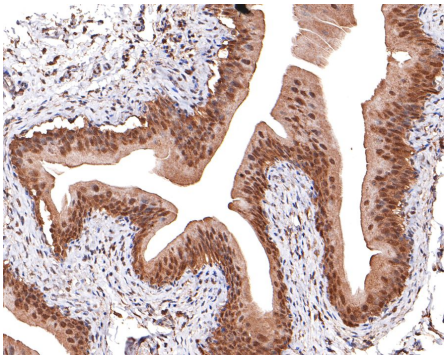

Fig3: Immunohistochemical analysis of paraffin-embedded rat bladder tissue with Rabbit anti-Cystatin-B antibody (HA720084) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA720084) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

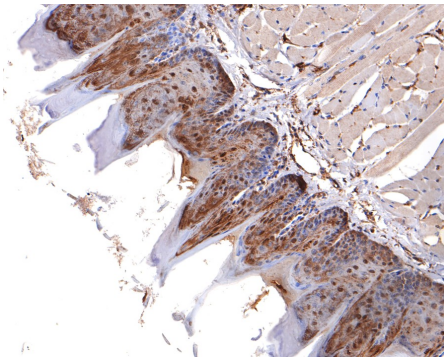

Fig4: Immunohistochemical analysis of paraffin-embedded rat tongue tissue with Rabbit anti-Cystatin-B antibody (HA720084) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA720084) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

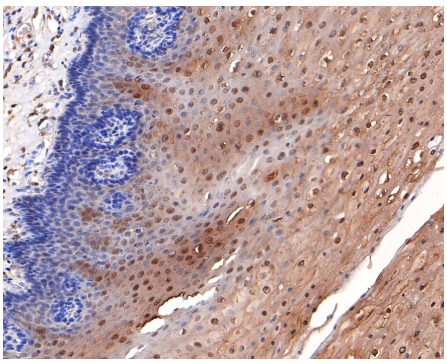

Fig5: Immunohistochemical analysis of paraffin-embedded human esophagus tissue with Rabbit anti-Cystatin-B antibody (HA720084) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA720084) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

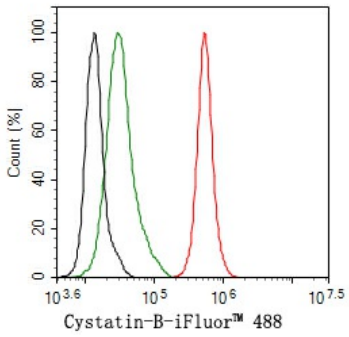

Fig6: Flow cytometric analysis of PC-3M cells labeling Cystatin-B.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA720084, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Wickramasinghe PDSU. et. al. Identification and characterization of cystatin B from black rockfish, Sebastes schlegelii, indicating its potent immunological importance. Fish Shellfish Immunol. 2020 Sep
2. Di Matteo F. et. al. Cystatin B is essential for proliferation and interneuron migration in individuals with EPM1 epilepsy. EMBO Mol Med. 2020 Jun