



Monoclonal Anti-human Cystatin B Antibody

ORDERING INFORMATION

Catalog Number: MAB1408

Clone: 225228

Lot Number: JUV02

Size: 500 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human Cystatin B

Immunogen: *E. coli*-derived rhCystatin B

Ig class: mouse IgG_{2b}

Recommended Applications:

Neutralization
Western blot
Immunoprecipitation

Other Application:

Direct ELISA

Background

Cystatin B is an intracellular inhibitor of cysteine proteases such as Cathepsins B, H, L and S. Mutations in the Cystatin B gene are the cause of progressive myoclonus epilepsy.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived, recombinant human Cystatin B (rhCystatin B; aa 2 - 98; Accession # P04080). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody detects rhCystatin B in Western blots and direct ELISAs. In Western blots, this antibody showed less than 5% cross-reactivity with rhCystatins A and S, rmCystatin B, rhFetuin A and rhHPRG (Histidine-Proline-Rich Glycoprotein) and approximately 5% to 10% cross-reactivity with rhCystatins C, D, E/M, SA and SN and rhFetuin B.

Applications

Neutralization - This antibody was used to neutralize the inhibitory activity of rhCystatin B (R&D Systems, Catalog # 1408-PI) against papain. The antibody was preincubated with the inhibitor at different molar ratios at room temperature (RT) for 30 minutes. The resulting mixtures were then incubated with the enzyme at RT for 15 minutes. The enzyme was then assayed with a substrate, Z-F-R-AMC. 50% of the proteolytic activity was restored by the antibody at 5.4 µg/mL (IC₅₀) under conditions in which 0.53 µg/mL of the inhibitor, 0.1 µg/mL of the enzyme, and 100 µM of the substrate were present. Considering the molecular masses of the inhibitor (12 kDa) and the antibody (150 kDa), IC₅₀ was achieved at approximately 1:1 molar ratio of the antibody to the inhibitor.

Western blot - This antibody can be used at 1 - 2 µg/mL with the appropriate secondary reagents to detect human Cystatin B. Using a colorimetric detection system, the detection limit for rhCystatin B is approximately 25 ng/lane under non-reducing and reducing conditions. Chemiluminescent detection will increase sensitivity by 5 to 50 fold.

Immunoprecipitation - This antibody was used at 25 µg/mL to immunoprecipitate rhCystatin B from conditioned cell culture media.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human Cystatin B. The detection limit for rhCystatin B is approximately 10 ng/well.

Optimal dilutions should be determined by each laboratory for each application.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

1/8/09