

ORDERING INFORMATION

Catalog Number: AF1286

Lot Number: JSX01

Size: 100 μg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human Cystatin E/M

Immunogen: NS0-derived rhCystatin E/M (aa 29 - 149)

Ig Type: specific goat IgG

Applications: Direct ELISA Western blot Neutralization Immunoprecipitation Immunohistochemistry

Anti-human Cystatin E/M Antibody

Preparation

Produced in goats immunized with purified, NS0-derived, recombinant human Cystatin E/M (rhCystatin E/M; aa 29 - 149). Human Cystatin E/M specific IgG was purified by human Cystatin E/M 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 0.1 mg/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 has been selected for its ability to neutralize human Cystatin E/M inhibitory activity.

Applications

Direct ELISA - This antibody can be used at $0.5 - 1.0 \mu g/mL$ with the appropriate secondary reagents to detect human Cystatin E/M. The detection limit for rhCystatin E/M is approximately 0.5 ng/well.

Western blot - This antibody can be used at 0.1 - 0.2 μ g/mL with the appropriate secondary reagents to detect human Cystatin E/M. The detection limit for rhCystatin E/M is approximately 5 ng/lane under non-reducing and reducing conditions. In this format, this antibody shows approximately 25% cross-reactivity with rmCystatin E/M and less than 1% cross-reactivity with rhCystatin A, rhCystatin B, rhCystatin C, rhCystatin D, rhCystatin S, rhCystatin SA, rhCystatin SN, rhFetuin A and rhFetuin B.

Neutralization - This antibody has been used to neutralize the inhibitory activity of rhCystatin E/M (R&D Systems, Catalog # 1286-PI) against papain. The antibody was pre-incubated with the inhibitor at different molar ratios at 37° C for 30 minutes. The resulting mixtures were then incubated with the enzyme at 37° C for 30 minutes. The enzyme was assayed with a substrate, Z-F-R-AMC (R&D Systems, Catalog # ES009). 50% of the proteolytic activity was restored by the antibody at 13 μ g/mL (IC₅₀) under conditions in which 1 μ g/mL of the inhibitor, 0.1 μ g/mL of the enzyme, and 100 μ M of the substrate were present. Considering the molecular masses of the inhibitor (15 kDa) and the antibody (150 kDa), IC₅₀ was achieved at approximately 1:1 molar ratio of the antibody to the inhibitor.

Immunoprecipitation - This antibody has been used to immunoprecipitate rhCystatin E/M from conditioned media of transfected NS0 cells. The recovered rhCystatin E/M can be detected by western blot analysis using a monoclonal antibody (R&D Systems, Catalog # MAB1286).

Immunohistochemistry - This antibody will detect Cystatin E/M in cells and tissues. The working dilution is 2 - 5 μ g/mL. For chromogenic detection of labeling, use R&D Systems' Cell and Tissue Staining Kits (CTS Series).

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

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