



cell signaling solutions

Certificate of Analysis

48 Barn Road • Lake Placid, NY 12946

Technical Support: T: 800 548-7853 • F: 518 523-4513

email: techserv@upstate.com

Sales Department: T: 800 233-3991 • F: 781 890-7738

Licensing Dept.: 800 310-4659

www.upstate.com

Anti-Cystatin C

(rabbit polyclonal IgG)

Catalog # 06-458

Lot # 29631

Immunogen: Human Cystatin C isolated from the urine of a patient with tubular proteinuria.

Specificity: Cystatin C at a Mr of 14kDa and its precursor.

Species Cross Reactivity: Mouse and rat.

Formulation: 250µg of DEAE purified rabbit IgG that has been depleted of antibodies reactive with human plasma proteins in 250µl PBS, pH 7.4 containing 0.05% sodium azide. Frozen solution.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of product, centrifuge the vial after thawing and prior to removing the cap.

**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing and Research Applications

Immunoblot Analysis: 1-2µg/ml of this lot detected Cystatin C in mouse spleen, thymus, and brain extracts.

Immunohistochemistry: 10µg/ml of a previous lot detected Cystatin C in paraformaldehyde-fixed rat brain section.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 μ g/ml aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Wash the blotted nitrocellulose with PBS-0.05% Tween for 10 minutes.
3. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), 0.05% Tween 20 (PBST-MLK) for 45 minutes at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-Cystatin C**, diluted in freshly prepared PBST-MLK overnight with agitation at 4°C.
5. Wash the nitrocellulose twice with water.
6. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit** HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBST-MLK for 1.5 hours at room temperature with constant agitation.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice (enhanced chemiluminescence was used).

Immunohistochemistry

1. Wash the tissue three times for 5 minutes with PBS.
2. Add fix (ice-cold 4% paraformaldehyde) in PBS for 1 minute at room temperature.
3. Wash the tissue with PBS, twice, for 15 minutes. Do not shake.
4. Add 400 μ l of 0.08% albumin in PBS and incubate for 30 minutes at room temperature.
5. Wash the tissue with PBS, for 15 minutes.
6. Incubate the tissue with **10 μ g/ml of anti-Cystatin C** in 0.08% albumin in PBS and incubate overnight at 4°C.
7. Wash the tissue twice with PBS, for 5 minutes.
8. Incubate the tissue with a **1:100 dilution of goat anti-rabbit IgG** fluorescein conjugated secondary antibody in PBS for 1 hour at room temperature.
9. Wash the tissue three times with PBS, for 5 minutes.
10. Examine the tissue under a fluorescent microscope.