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Certificate of Analysis

Anti-Cystatin C
(rabbit polyclonal IgG)
Catalog # 06-458
Lot # 21767

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

Specificity: Cystatin C and its precursor.

Species Cross Reactivity: Mouse and rat.

Formulation: 250mg of DEAE purified rabbit IgG in 250ml 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

Western Immunoblot Analysis: 1-2µg/ml of this lot detected Cystatin C in serum-treated mouse SFME cell lysates.

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

Western 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 20-25°C with constant agitation.
4. Incubate the nitrocellulose with **1-2mg/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 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 **10mg/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.