upstate biotechnology

Certificate of Analysis

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Anti-Human Cystatin C
(rabbit polyclonal IgG)

Catalog # 06-458 **Lot # 14013**

Background: As an inhibitor of cysteine proteinases, this protein is thought to serve an important physiological role as a local regulator of this enzyme activity.

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

Physical Form: Frozen liquid.

Formulation: **250µg** DEAE-purified IgG in **250µl** PBS pH 7.4.

Specificity: Cystatin C and its precursor.

Species Cross Reactivity: Mouse and rat.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing and Research Applications

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

Immunohistochemistry: 10µg/ml of this lot detected Cystatin C in 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; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk, 0.05% Tween 20 (PBS-MLK-T) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **2µg/ml of a-Human Cystatin C**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C overnight.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit** IgG linked to horseradish peroxidase, 1:3000 dilution, was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunocytochemistry

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 400µl 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 **10µg/ml of a-Human 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.