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Certificate of Analysis

Anti-Cystatin C
(rabbit polyclonal IgG)
Catalog # 06-458
Lot # 32621

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

Specificity: Recognizes Cystatin C, 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 of storage buffer (PBS, pH 7.4, 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

Immunoblot Analysis: 0.5-2µg/ml of this lot detected Cystatin C in mouse brain extract. A previous lot detected Cystatin C in mouse spleen and thymus extracts.

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

Additional Research Applications

ELISA: Recommended.

Application References:

1. Simonsen, O., *et al.*, Scan J. Clin. Invest. **45**: 97-101, 1985.
2. Pergrande, M. & Jung, K., Clin. Chem. **39**: 1885-1890, 1993.

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 EDTA; 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 TBS-0.05% Tween[®]-20 for 10 minutes.
3. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween[®]-20 (TBST-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 TBST-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, 1:3000 dilution was used) in TBST-MLK for 1.5 hours at room temperature with constant agitation.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in TBS-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 in the dark 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.