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## Certificate of Analysis

**Anti-Cystatin C**  
(rabbit polyclonal IgG)  
Catalog # 06-458  
Lot # JBC1355777

**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**

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### 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.

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### Additional Research Applications

ELISA: Recommended.

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### 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; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 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<sup>®</sup>-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<sup>®</sup>-20 (TBST-MLK) for 45 minutes at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.5-2µg/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<sup>®</sup>-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 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 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.