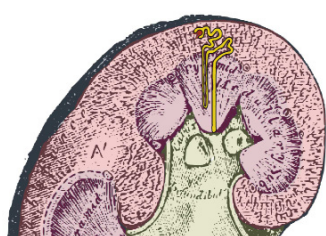


Human Cystatin C



Cystatin C is a low molecular weight (13.4 kDa) protein that functions as an inhibitor of various cysteine proteases in the bloodstream. It inhibits both endogenous proteases, such as lysosomal cathepsins, and proteases of parasites and microorganisms.

Cystatin C binds to the target molecule in μM to the sub pM range in a competitive reversible manner (1). Due to its important function, cystatin C is expressed at the stable levels by most of the nucleated cells. Cystatin C consists of 120 amino acid residues encoded by a 7.3 kb gene located in chromosome 20 (2). The Leu68Gln mutation in the cystatin C protein sequence is directly linked to the development of hereditary cystatin C amyloid angiopathy (HCCAA) in which the patients suffer from repeated cerebral hemorrhages (3).

Cystatin C is known in clinical practice as a well-described serum marker of renal failure that is not dependent on age, sex or lean muscle mass (4, 5). At the same time, cystatin C is becoming acknowledged as a marker of elevated risk of death from cardiovascular complications – myocardial infarction and stroke (5). A stable production rate and free filtration by the renal glomeruli due to the low molecular weight, and positive charge (pI 9.3) are strong advantages of cystatin C as a serum marker of renal function in comparison to other analytes that are used today in clinical practice. Creatinine-based equations to estimate the glomerular filtration rate (GFR) are sensitive to some non-renal factors, such as age, sex, race and lean muscle mass. There is a growing number of reports demonstrating that cystatin C is more preferable than creatinine for the measurement of GFR, so long as it does not depend on all of these factors (5).

CLINICAL UTILITY

- Measurement glomerular filtration rate
- Marker of renal dysfunction and failure.
- Marker of elevated risk of death from cardiac diseases and stroke

Cystatin C is also a more sensitive marker of mild renal dysfunction than creatinine (6). The concentrations of plasma (serum) cystatin C in healthy individuals range from 0.8 to 1.2 mg/l, depending on measurement methods (7). Increased cystatin C serum levels are almost exclusively associated with a reduction in GFR. Serum concentrations of cystatin C are increased approximately 2-fold during various renal disorders (7). An elevated serum cystatin C level is also a strong predictor of the risk of death and cardiovascular events in elderly persons (5).

The urinary concentrations of cystatin C are low (100 $\mu\text{g/l}$ for healthy subjects) since the protein is metabolized by the proximal tubule after filtration in the renal glomerulus. However, the concentrations of cystatin C in urine from patients with renal tubular disorders are raised by approximately 200-fold (8). Cystatin C that is purified from human urine can be partially truncated, which potentially complicates the application of the urine protein as a standard for immunoassays(9).

Advanced ImmunoChemical offers high affinity products for the development of the cystatin C immunoassay - human recombinant cystatin C, native human cystatin C purified from human blood, anti-cystatin C polyclonal antibodies, as well as a set of high-affinity monoclonal antibodies that are specific to different epitopes of human cystatin C molecule. We also supply information regarding the best MAb combinations to be used in sandwich immunoassays for quantitative measurements of cystatin C in body fluids.

Human cystatin C antigens

Advanced ImmunoChemical offers recombinant human cystatin C expressed in *E. coli* as a full length peptide with additional methionine residue at the N-terminus. The protein is purified to homogeneity using several chromatography methods (Fig. 1).

Immunochemical properties of human recombinant cystatin C expressed in *E. coli*, cystatin C purified from pooled human serum, and cystatin C purified from human urine (RDI) were analyzed by seven prototype cystatin C immunoassays (Fig. 2).

Human recombinant cystatin C and cystatin C purified from pooled human serum had very similar immunochemical activity with the antigen in human serum in cases of all tested assays. However, cystatin C purified from human urine had significantly lower immunochemical activity when measured by four out of seven tested immunoassays. It can be explained by possible truncation of cystatin C purified from human urine. This data suggests that recombinant and purified antigens from human blood serve better as standards or calibrators in cystatin C immunoassays than protein purified from human urine.

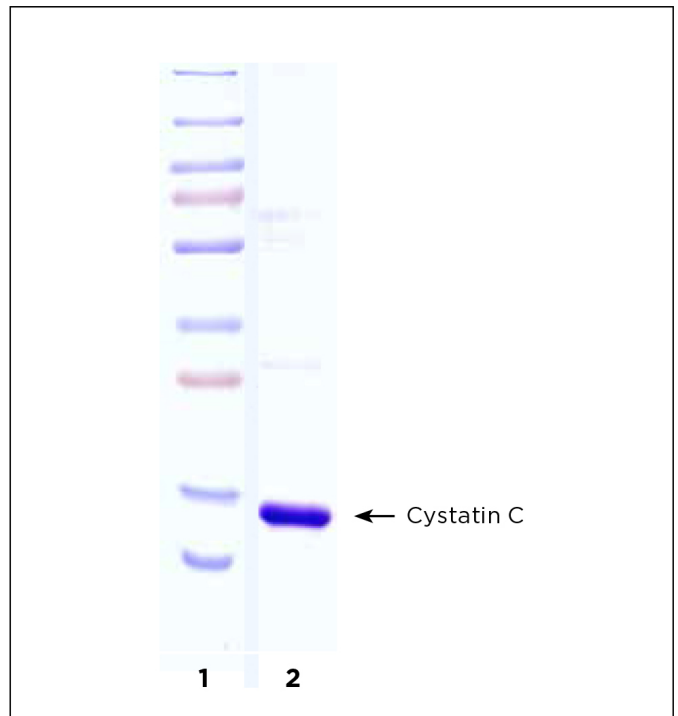


Figure 1. SDS-PAGE of human recombinant cystatin C expressed in *E. coli*, reducing conditions.

Lane 1: Molecular weight standards, Fermentas (250, 130, 92, 75, 55, 36, 28, 17, and 11 kDa)

Lane 2: Human recombinant cystatin C from *E. coli*, 5 µg. Gel staining: Coomassie brilliant blue R-250.

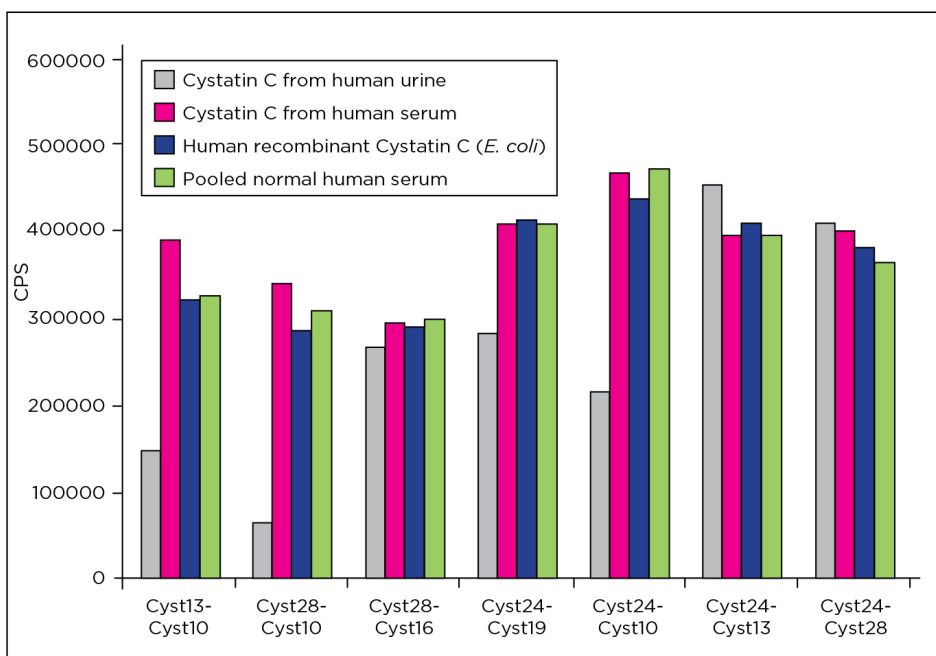


Figure 2. Immunochemical properties of three forms of cystatin C protein, in comparison with antigen from pooled normal human serum.

Cystatin C preparations (all at concentration 10 ng/ml) and diluted pooled normal human serum were analyzed.

Sandwich type fluoroimmunoassay was used to measure cystatin C:

Capture MAbs: Cyst13, Cyst28 and Cyst24.

Detection MAbs: Cyst10, Cyst16, Cyst13, Cyst19 and Cyst28 are Eu³⁺-labeled.

Monoclonal antibodies specific to cystatin C

Hybridoma clones have been derived from the hybridization of Sp2/0 myeloma cells with spleen cells of Balb/c mice immunized with cystatin C purified from human urine. Eight anti-cystatin C MAbs were selected in regard to their specificity and high-affinity interaction with the cystatin C molecule.

Cystatin C immunodetection in Western blotting

Several monoclonal antibodies – Cyst13, Cyst18 and Cyst19 – could be used for cystatin C immunodetection in Western blotting (Fig. 3).

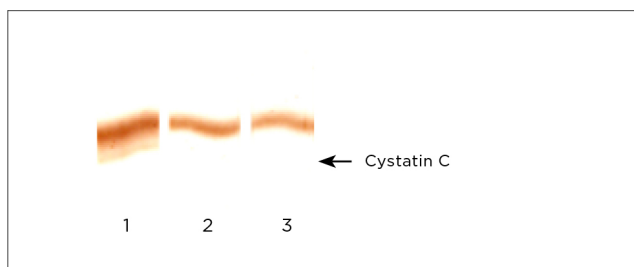


Figure 3. Detection of human cystatin C in Western blotting by different monoclonal antibodies after Tricine-SDS-PAGE in reducing conditions.

Lane 1: MAb Cyst13

Lane 2: MAb Cyst18

Lane 3: MAb Cyst19

Antigen: Cystatin C purified from human urine (RDI), 0.2 µg/lane.

Cross-reaction with different animal species

Among all possible sandwich combinations of anti-cystatin C MAbs produced by using human antigen, the set of pairs with significant cross-reactivity with dog, cat or horse serum (Table 1) is shown below.

	Dog	Cat	Horse
Cyst29 - Cyst11	+	+	-
Cyst29 - Cyst16	+	++	-
Cyst11 - Cyst20	++	+	-
Cyst29 - Cyst20	+	++	++
Cyst11 - Cyst29	+	+	-
Cyst16 - Cyst29	+	+	-
Cyst20 - Cyst29	-	+	++
Cyst20 - Cyst13	-	-	++
Cyst29 - Cyst13	-	-	++

Table 1. Cross-reaction of anti-cystatin C MAbs with sera from different animal species. Sandwich type fluoroimmunoassay was used to measure cross-reaction; capture-detection MAb pairs are shown in the table. No cross-reaction (-), 7-30% cross-reaction (+), or 30-90% cross-reaction (++) are indicated in comparison with pooled normal human serum.

Cystatin C quantitative sandwich immunoassays

All selected MAbs were tested in sandwich fluoroimmunoassay as capture and detection antibodies with purified human antigen and pooled serum samples (Fig. 4 and 5). The best recommended pairs (capture - detection) are:

Cyst24 – Cyst19

Cyst24 – Cyst28

Cyst23 – Cyst13

These pairs demonstrate high sensitivity and perfect antigen recognition in blood samples.

The best MAb combinations can be used for antigen detection even at 100,000-fold serum dilution (Fig. 5). For these assays a high degree of parallelism between titration curve of purified human cystatin C and the curves of serial dilutions of pooled serum sample was observed.

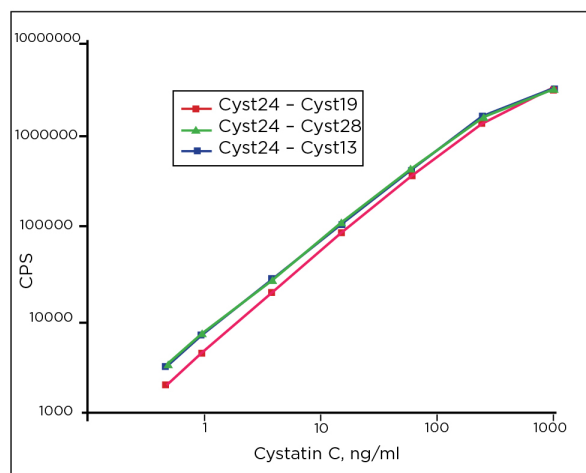


Figure 4. Calibration curves of the best immunoassays.

One-step fluoroimmunoassay in streptavidin coated plates.

Capture MAbs Cyst24 and Cyst23 are biotinylated (200 ng/well). Detection MAbs Cyst19, Cyst28 or Cyst13 are Eu3+-labeled (200 ng/ml). Incubation volume 100 µl. time: 30 min at room temperature.

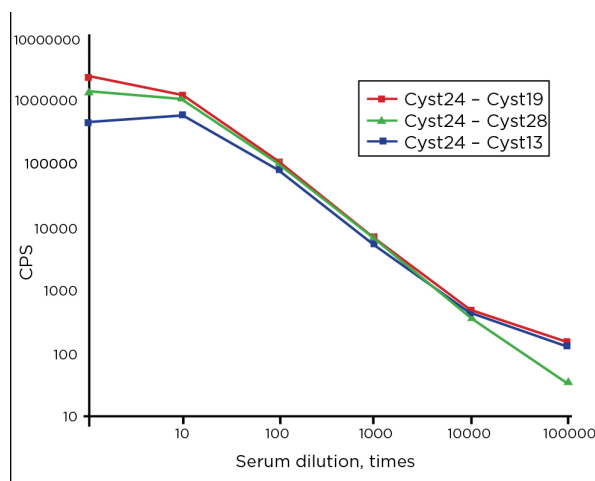


Figure 5. Titration curves of pooled normal human serum in Cyst24 – Cyst19, Cyst24 – Cyst28, and Cyst23 – Cyst13 (capture – detection) sandwich fluoroimmunoassays.

Polyclonal anti-cystatin C antibodies

Polyclonal anti-cystatin C antibodies were obtained through the immunization of sheeps with highly purified (>95%) human recombinant cystatin C expressed in *E. coli*. Affinity chromatography utilizing human recombinant cystatin C-sepharose makes it possible to produce highly purified anti-cystatin C polyclonal antibodies that are free from sheep serum proteins and non-specific immunoglobulins.

Cystatin C free serum

Cystatin C free serum is prepared from pooled normal human serum by immunoaffinity chromatography method. Cystatin C free serum can be used as a matrix for standard and calibrator preparation.

Ordering Information:

MONOCLONAL ANTIBODIES

Product	Cat #	MAb	Subclass	Remarks
Cystatin C	2-CYSC	Cyst10	1gG3	EIA
		Cyst11	1gG1	EIA, C/r with dog and cat serum
		Cyst13	1gG1	EIA, WB, C/r with horse serum
		Cyst16	1gG1	EIA, WB, C/r with horse serum
		Cyst18	1gG1	EIA, WB
		Cyst19	1gG1	EIA, WB
		Cyst20	1gG1	EIA, C/r with dog, cat and horse serum
		Cyst23	1gG1	EIA
		Cyst24	1gG1	EIA
		Cyst28	1gG1	EIA
		Cyst29	1gG2a	EIA, C/r with dog, cat and horse serum

POLYCLONAL ANTIBODY

Product	Cat #	Host Animal	Remarks
Cystatin C	6-CYS	Sheep	EIA, WB, IP

ANTIGEN

Product	Cat #	Purity	Source
Cystatin C, human, recombinant	8-CYC-rh	>95%	Recombinant
Cystatin C, human	8-CYC	>95%	Pooled human serum, Available only in research amounts

DEPLETED SERUM

Product	Cat #	Host Animal
Cystatin C free serum	11-CYC-fs	Pooled normal human serum

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