Human Cystatin C

Cystatin C is a lowmoleclar weight(13.4 kDa) protein that functions as an inhibitor of various cysteine proteases in the bloodstream. Itinhibits both endo-genous proteases, such as lysosomal cathepsins, and pro-teases 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).

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 μg/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).

Hytest offers everything you need 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 our customers with 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

Hytest 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 Hytest prototype cystatin C immunoassays (Fig. 2).

Hytest's 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.

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. 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

MAbs Cyst13 and Cyst19cc could be used for cystatin C immunodetection in Western blotting (Fig. 3).

Cross-reaction with different animal species

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

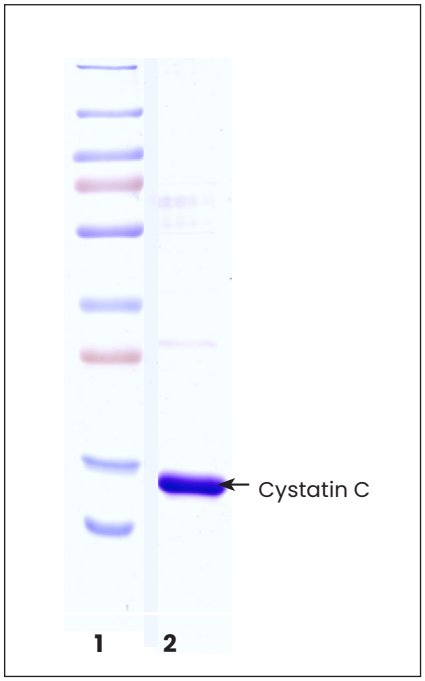

Figure 1.
SDS-PAGE of human recombinant cystatin C expressed in E. coli, reducing conditions.
Lane 1: Molecular weight standards, Fermentas (250, 130, 92, 75, 55, 36, 28, 17, and 11 kDa)
Lane 2: Human recombinant cystatin C from E. coli, 5 µg.
Gel staining: Coomassie brilliant blue R-250.

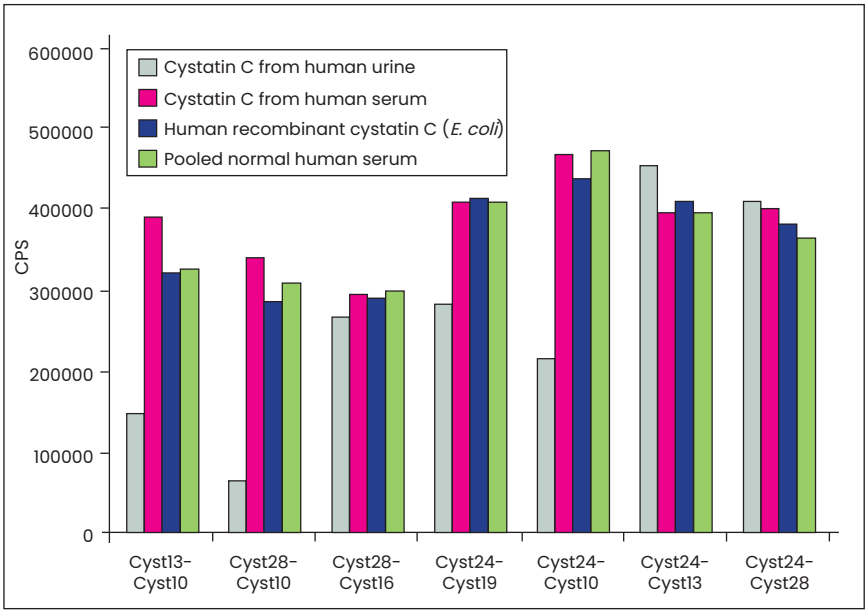

Figure 2.
Immunochemical properties of three forms of cystatin C protein, in comparison with antigen from pooled normal human serum.
Cystatin C preparations (all at concentration 10 ng/ml) and diluted pooled normal human serum were analyzed.
Sandwich type fluoroimmunoassay was used to measure cystatin C:
Capture MAbs: Cyst13, Cyst28 and Cyst24.
Detection MAbs: Cyst10, Cyst16, Cyst13, Cyst19 and Cyst28 are Eu^{3+}-labeled.

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:

Cyst24cc – Cyst19cc
Cyst24cc – Cyst28
Cyst23cc – 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 we observed high degree of parallelism between titration curve of purified human cystatin C and the curves of serial dilutions of pooled serum sample.

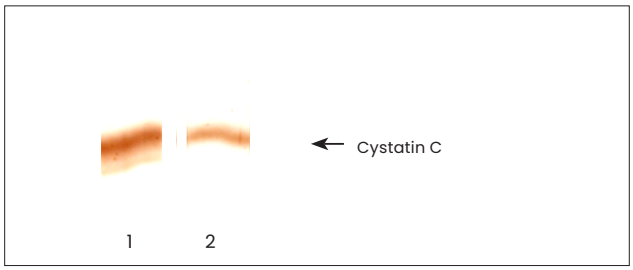

Figure 3.
Detection of human cystatin C in Western blotting by different monoclonal antibodies after Tricine-SDS-PAGE in reducing conditions.
Lane 1: MAb Cyst13
Lane 2: MAb Cyst19
Antigen: Cystatin C purified from human urine (RDI), 0.2 µg/lane.

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.*

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

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.

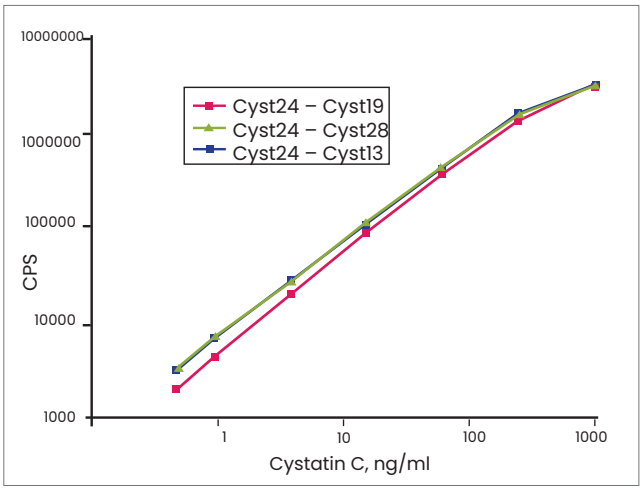

Figure 4.
Calibration curves of the best immunoassays.
One-step fluoroimmunoassay in streptavidin coated plates.
Capture MAbs Cyst24 and Cyst23 are biotinylated (200 ng/well).
Detection MAbs Cyst19, Cyst28 or Cyst13 are Eu^{3+}-labeled (200 ng/ml).
Incubation volume 100 µl. time: 30 min at room temperature.

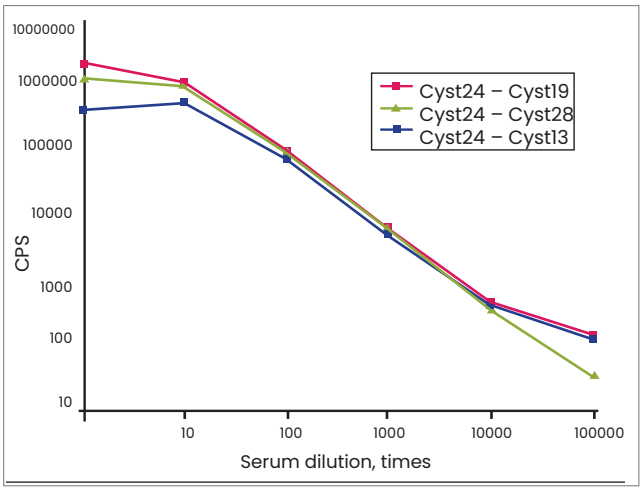

Figure 5.
Titration curves of pooled normal human serum in Cyst24–Cyst19, Cyst24–Cyst28, and Cyst23–Cyst13 (capture–detection) sandwich fluoroimmunoassays.

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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Cystatin C	4CC1	Cyst10	IgG3	EIA
		Cyst11	IgG1	EIA, C/r with dog and cat serum
		Cyst13	IgG1	EIA, WB, C/r with horse serum
		Cyst16	IgG1	EIA, C/r with dog and cat serum
		Cyst19cc	IgG1	*In vitro*, EIA, WB
		Cyst20	IgG1	EIA, C/r with dog, cat and horse serum
		Cyst23	IgG1	EIA
		Cyst24cc	IgG1	*In vitro*, EIA
		Cyst28	IgG1	EIA
		Cyst29	IgG2a	EIA, C/r with dog, cat and horse serum

POLYCLONAL ANTIBODY

Product name	Cat. #	Host Animal	Remarks
Cystatin C	PCC2	sheep	EIA, WB, IP

ANTIGEN

Product name	Cat. #	Purity	Source
Cystatin C, human, recombinant	8CY5	>95%	Recombinant

DEPLETED SERUM

Product name	Cat. #	Source
Cystatin C free serum	8CCFS	Pooled normal human serum

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *w.* MAbs produced *in vitro* are expected to have similar performance.

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