HyTest NEWS

Human cystatin C

Cystatin C is a low molecular weight (13.4 kDa) protein functioning 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 sub pM range in competitive reversible manner (1). Due to its important function cystatin C is expressed at the stable levels by most nucleated cells. Cystatin C consists of 120 amino acid residues encoded by a 7.3 kb gene located in chromosome 20 (2). 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).

In clinical practice cystatin C is known 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). 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 with other analytes used today in clinical practice. Creatinine-based equations to estimate the glomerular filtration rate (GFR) are sensitive to some nonrenal factors, such as age, sex, race and lean muscle mass. There is a growing number of reports demonstrating that cystatin C is more preferable to measure GFR than creatinine as long as it does not depend on all these factors (5). Cystatin C is also more sensitive marker of mild renal dysfunction than creatinine (6). The concetrations 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 about 2-fold during various renal disorders (7). 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 approximately 200-fold (8). Cystatin C purified from human urine can be partially truncated, that potentially complicates the application of the urine protein as a standard for immunoassays (9).

HyTest offers everything you need for the development of cystatin C immunoassay - human recombinant cystatin C (***New***!), native human cystatin C purified from human blood (***New***!), anti-cystatin C polyclonal antibodies (***New***!) as well as a set of high-affinity monoclonal antibodies specific to different epitopes of human cystatin C molecule. We also supply our customers with information about the best MAb combinations to be used in sandwich immunoassays for quantitative measurements of cystatin C in body fluids.

Human cystatin C antigens *New!*

1. Human recombinant cystatin C

Source:	Recombinant, expressed in *E. coli*
Purity:	>95 %
Presentation:	Lyophilized
Application:	Standard or calibrator for cystatin C immunoassays, cystatin C biochemical and immunochemical studies
Storage:	-20°C

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).

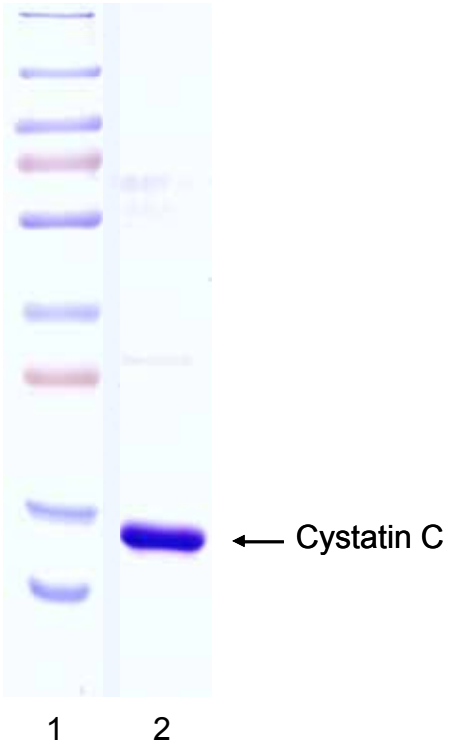

Figure 1. SDS-PAGE of human recombinant cystatin C expressed in *E. coli*, reducing conditions.
Lane 1: Molecular weight standards, Fermentas (250, 130, 92, 75, 55, 36, 28, 17, and 11 kDa)
Lane 2: Human recombinant cystatin C from *E. coli*, 5 µg.
Gel staining: Coomassie brilliant blue R-250.

2. Cystatin C purified from human blood

Source:	Pooled human serum
Purity:	>95 %
Presentation:	Lyophilized
Application:	Standard or calibrator for cystatin C immunoassays, cystatin C biochemical and immunochemical studies
Storage:	-20°C

HyTest offers native human cystatin C purified from pooled normal human serum. The protein is purified to homogeneity using several chromatography methods (Fig. 2).

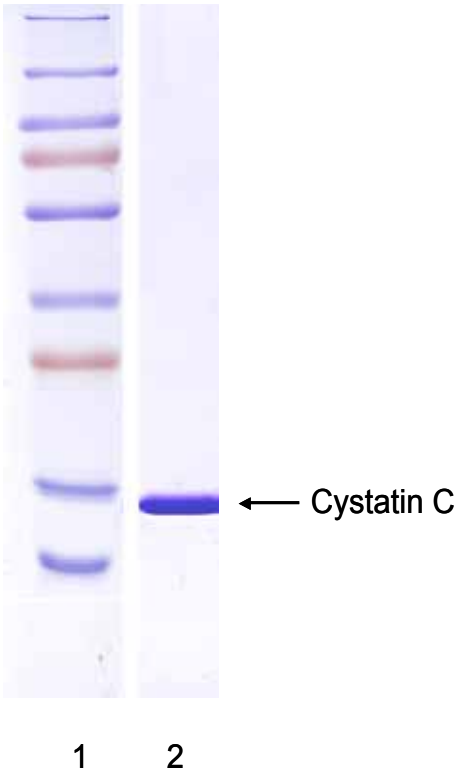

Figure 2. SDS-PAGE of cystatin C from pooled human serum, reducing conditions.
Lane 1: Molecular weight standards, Fermentas (250, 130, 92, 75, 55, 36, 28, 17, and 11 kDa)
Lane 2: Cystatin C from pooled human serum, 5 µg.
Gel staining: Coomassie brilliant blue R-250.

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 analysed by seven HyTest prototype cystatin C immunoassays (described in details in Part 2 of this Newsletter) (Fig. 3).

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 case 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. These data suggest that recombinant and purified from human blood antigens serve better as standards or calibrators in cystatin C immunoassays than protein purified from human urine.

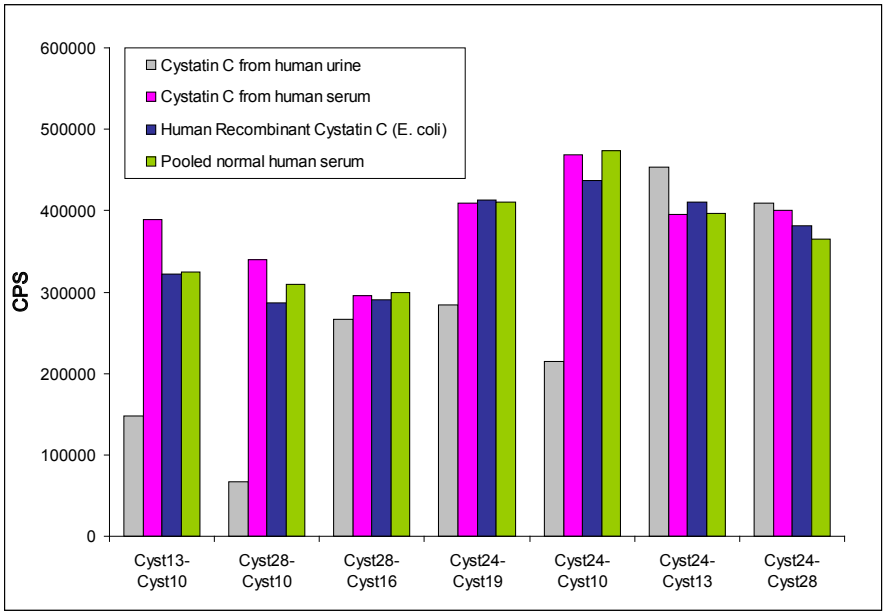

Figure 3. Immunochemical properties of three forms of cystatin C protein, in comparison with antigen from pooled normal human serum.
Cystatin C preparations (all at concentration 10 ng/ml) and diluted pooled normal human serum were analyzed.
Sandwich type fluoroimmunoassay was used to measure cystatin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

Ordering information:

Product	Cat.#	Purity	Source
Cystatin C, human, recombinant	8CY5	>95%	*E. coli*
Cystatin C, human, endogenous	8CN4	>95%	Pooled human serum

Anti-cystatin C monoclonal antibodies

Host animal:	Mice Balb/c
Cell line used for fusion:	Sp2/0
Antigen:	Human cystatin C
Purification method:	Protein A affinity chromatography
Presentation:	MAb solution in PBS with 0.1 % sodium azide
Application:	Cystatin C immunoassays, cystatin C immunodetection in Western blotting

Hybridoma clones have been derived from 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 respect to their specificity and high-affinity interaction with cystatin C molecule.

Applications

1. Cystatin C quantitative sandwich immunoassays

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

Cyst24 – Cyst10
Cyst23 – Cyst10
Cyst24 – Cyst19
Cyst24 – Cyst28
Cyst23 – Cyst13

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

The best MAb combination (Cyst24 – Cyst10) can be used for antigen detection even at 100,000-fold serum dilution (Fig. 5). For this assay we observed the highest degree of parallelism between titration curve of purified human cystatin C and a curve of serial dilutions of pooled serum sample.

Affinity constants for Cyst10 and Cyst24 MAbs (human cystatin C from RDI used as an antigen) were measured using Biacore technique:

Cyst24 - 1.09×10^{-8} M
Cyst10 - 5.55×10^{-8} M

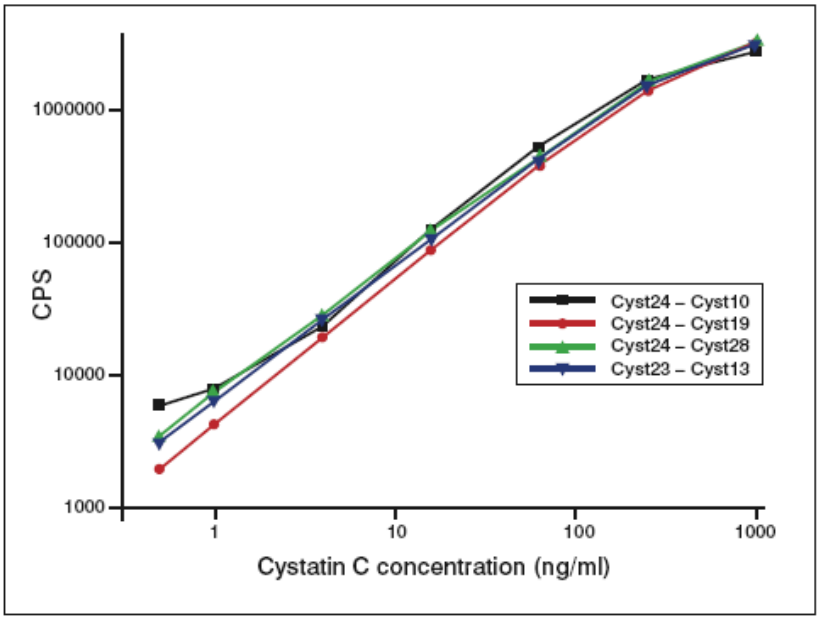

Figure 4. Calibration curves of the best immunoassays. One-step fluoroimmunoassay in streptavidin coated plates.
Capture MAbs: Cyst24 and Cyst23 are biotinylated (200 ng/well).
Detection MAbs: Cyst10, Cyst19, Cyst28 or Cyst13 are Eu^{3+}-labelled (200 ng/ml).
Incubation volume 100 μl.
Incubation time: 30 min at room temperature.

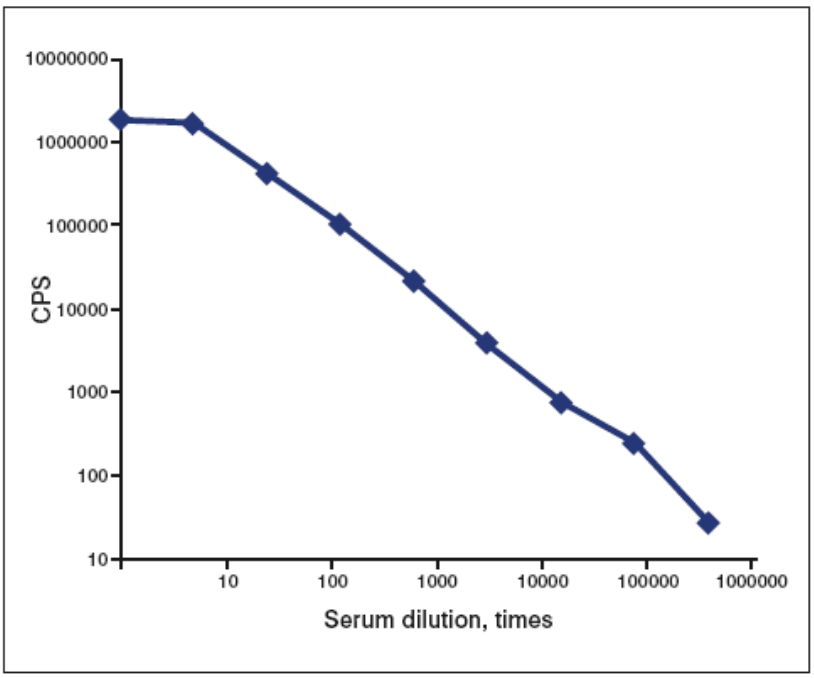

Figure 5. Titration curve of pooled normal serum in Cyst24 – Cyst10-Eu^{3+} sandwich fluoroimmunoassay.

2. Cystatin C immunodetection in Western blotting

Several monoclonal antibodies – Cyst13, Cyst18 and Cyst19 – could be used for cystatin C immunodetection in Western blotting (Fig. 6).

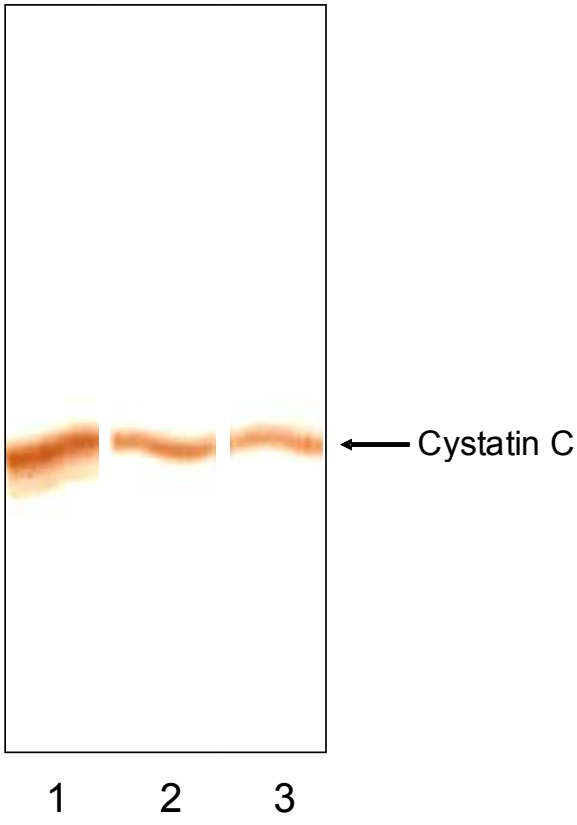

Figure 6. Detection of human cystatin C in Western blotting by different monoclonal antibodies after Tricine-SDS-PAGE in reducing conditions.
Lane 1: MAb Cyst13
Lane 2: MAb Cyst18
Lane 3: MAb Cyst19
Antigen: Cystatin C purified from human urine (RDI), 0.2 µg/lane.

Ordering information:

Product	Cat.#	MAb	Subclass	Application
Anti-cystatin C	4CC1	Cyst10	IgG3	EIA (capture)
Anti-cystatin C	4CC1	Cyst13	IgG1	EIA (detection), WB
Anti-cystatin C	4CC1	Cyst16	IgG1	EIA
Anti-cystatin C	4CC1	Cyst18	IgG1	EIA, WB
Anti-cystatin C	4CC1	Cyst19	IgG1	EIA (detection), WB
Anti-cystatin C	4CC1	Cyst23	IgG1	EIA (capture)
Anti-cystatin C	4CC1	Cyst24	IgG1	EIA (capture)
Anti-cystatin C	4CC1	Cyst28	IgG1	EIA (capture, detection)

Polyclonal anti-cystatin C antibodies *New!*

Host animal: Rabbit
Antigen: Human recombinant cystatin C
Purification method: Affinity chromatography utilizing human recombinant cystatin C-agarose
Presentation: PAb solution in PBS with 0.1 % sodium azide

Polyclonal anti-cystatin C antibodies were obtained by immunization of rabbits with highly purified (>95%) human recombinant cystatin C expressed in *E. coli*. Affinity chromatography utilizing human recombinant cystatin C-agarose makes it possible to produce highly purified anti-cystatin C polyclonal antibodies free from rabbit serum proteins and non-specific immunoglobulins

Ordering information:

Product	Cat.#	Host	Remarks
Polyclonal anti-cystatin C	PCC2	Rabbit	EIA, WB, IHC, IP

Cystatin C free serum

Source: Pooled normal human serum
Purification method: Immunoaffinity chromatography
Delivery form: Frozen liquid

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.

Serum sample	Cystatin C concentration (ng/ml)
Pooled serum (before cystatin C depletion)	800
Cystatin C free serum	0.4

Ordering information:

Product	Cat.#	Source
Cystatin C free serum	8CCFS	Pooled Normal Human Serum

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