# DRG® Cystatin C (human) ELISA (EIA-4394)

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***Please use only the valid version of the package insert provided with the kit.***

## 1 INTENDED USE

The Cystatin C (human) ELISA is a sandwich enzyme immunoassay for measurement of human cystatin C

This kit is intended for Research Use Only. Not for use in diagnostic procedures.

**Features**

- The total assay time is less than 2 hours.
- The kit measures total cystatin C in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid.
- Assay format is 96 wells.
- Quality Controls are human serum or human urine native protein based. No animal sera are used.
- Standard is purified native protein based.
- Components of the kit are provided ready to use or concentrated.
- Convenient for automatization.

## 2 STORAGE, EXPIRATION

Store the complete kit at 2°C - 8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

## 3 TEST PRINCIPLE

In the Human Cystatin C ELISA, standards, quality controls and samples are incubated in microtitrate plate wells pre-coated with polyclonal anti-human cystatin C antibody.

After 30 minutes incubation and washing, polyclonal anti-human cystatin C antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 30 minutes with captured cystatin C. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured

The absorbance is proportional to the concentration of cystatin C.

A standard curve is constructed by plotting absorbance values against concentrations of cystatin C standards, and concentrations of unknown samples are determined using this standard curve.

## 4 PRECAUTIONS

- **For professional use only.**
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.

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- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.

## 5 TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed.
- Use thoroughly clean glassware.
- Use deionized (distilled) water, stored in clean containers.
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent.
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light.
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

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## 6 REAGENT SUPPLIED

| Kit Components | State | Quantity |
|---|---|---|
| Antibody Coated Microtiter Strips | ready to use | 96 wells |
| Conjugate Solution Conc. (50x) | concentrated | 0.26 ml |
| Conjugate Diluent | ready to use | 13 ml |
| Set of Standards | concentrated | 6 x 0.1 ml |
| Quality Control HIGH | concentrated | 0.1 ml |
| Quality Control LOW | concentrated | 0.1 ml |
| Dilution Buffer Conc. (10x) | concentrated | 10 ml |
| Wash Solution Conc. (10x) | concentrated | 100 ml |
| Substrate Solution | ready to use | 13 ml |
| Stop Solution | ready to use | 13 ml |
| Product Data Sheet + Certificate of Analysis | | 1 pc |

## 7 MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650nm)
- Software package facilitating data generation and analysis (optional)

## 8 PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

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**Assay reagents supplied ready to use:**

**Antibody Coated Microtiter Strips**

*Stability and storage:*

Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

**Conjugate Diluent**

**Substrate Solution**

**Stop Solution**

*Stability and storage:* Opened reagents are stable 3 months when stored at 2-8°C.

**Assay reagents supplied concentrated:**

**Dilution Buffer Conc. (10x)**

Dilute Dilution Buffer Conc. (10x) ten-fold in 90 ml distilled water to prepare a 1x working solution, e.g. 10 ml of Dilution Buffer Conc. (10x) + 90 ml of distilled water for use of all 96-wells.

It is recommended to dilute only such a volume of Dilution Buffer Conc. (10x) to be used up in the one run of the test.

*Stability and storage:*

The diluted Dilution Buffer is stable 1 week when stored at 2-8°C.

Opened Dilution Buffer Conc. (10x) is stable 3 months when stored at 2-8°C.

**Set of Standards**

Dilute each concentration of Standard 400x with the Dilution Buffer just prior to the assay in two steps as follows:

**Dilution A** (10x):
Add 10 µl of Standard into 90 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

**Dilution B** (40x):
Add 10 µl of Dilution A into 390 µl of Dilution Buffer to prepare final dilution (400x). **Mix well** (not to foam). Vortex is recommended.

*Stability and storage:*

Opened Standards are stable 3 months when stored at 2-8°C.
**Do not store the diluted set of Standards.**

**Quality Controls High, Low**

**Refer to the Certificate of Analysis for current Quality Control concentration!!!**

Dilute each Quality Control (QC) 400x with the Dilution Buffer just prior to the assay in two steps as follows:

**Dilution A** (10x):
Add 10 µl of QC into 90 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

**Dilution B** (40x):
Add 10 µl of Dilution A into 390 µl of Dilution Buffer to prepare final dilution (400x). **Mix well** (not to foam). Vortex is recommended.

*Stability and storage:*

Opened Quality Controls are stable 3 months when stored at 2-8°C.

**Do not store the diluted Quality Controls.**

**It is recommended to supplement two or three negative sample controls of customer´s own** (in addition to those provided with this kit). They can serve as evidence of the difference between positive and negative samples (see Figure 5 and Figure 6).

**Conjugate Solution Conc. (50x)**

Prepare the working Conjugate Solution by adding 1 part concentrated Conjugate Solution Conc. (50x) with 49 parts Conjugate Diluent.

Example:
0.25 ml of Conjugate Solution Conc. (50x) + 12.25 ml of Conjugate Diluent for use of all 96-wells.
Prepare only the volume needed for the test. **Mix well** (not to foam).

*Stability and storage:*

Opened Conjugate Solution Conc. (50x) is stable 3 months when stored at 2-8°C.

**Do not store the diluted Conjugate Solution.**

**Wash Solution Conc. (10x)**

Dilute Wash Solution Conc. (10x) ten-fold in 900 ml of distilled water to prepare a 1x working solution,
e.g. 100 ml of Wash Solution Conc. (10x) + 900 ml of distilled water for use of all 96-wells.

*Stability and storage:*

The diluted Wash Solution is stable 1 month when stored at 2-8°C.
Opened Wash Solution Conc. (10x) is stable 3 months when stored at 2-8°C.

## 9 PREPARATION OF SAMPLES

The kit measures cystatin C in serum, plasma (EDTA, citrate, heparin), urine and cerebrospinal fluid.

Samples should be assayed immediately after collection or should be stored at -20°C.

Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples (serum, plasma) 400x with the Dilution Buffer just prior to the assay in two steps as follows:

**Dilution A** (10x):
Add 10 µl of sample into 90 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

**Dilution B** (40x):
Add 10 µl of Dilution A into 390 µl of Dilution Buffer to prepare final dilution (400x). **Mix well** (not to foam). Vortex is recommended.

Dilute samples (CSF) 1600x with the Dilution Buffer just prior to the assay as follows:

**Dilution A** (40x):

Add 10 µl of sample into 390 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

**Dilution B** (40x):

Add 10 µl of Dilution A into 390 µl of Dilution Buffer to prepare final dilution (1600x). **Mix well** (not to foam). Vortex is recommended.

*Stability and storage:*
Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
**Do not store the diluted samples.**

*Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.*

## 10 ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker. Incubation without shaking is the alternative that requires to extend incubation with substrate – see paragraph 8.
6. Wash the wells **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake with the plate during the incubation.

9. Stop the colour development by adding **100 µl** of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.
    **The absorbance should be read within 5 minutes following step 9.**

***Note:*** *If some samples and standards have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine cystatin C concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.*

***Note 2:*** *Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash* Solution has been removed entirely.

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*Figure 1: Example of a work sheet.*

## 11 CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of cystatin C ng/ml in samples.

Alternatively, the logit log function can be used to linearize the standard curve i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.
Use values of undiluted standard range: 10 000, 4 000, 2 000, 1 000, 400, 200 ng/ml.

**Samples, Quality Controls and Standards are all diluted 400x prior to analysis, so there is no need to take this dilution factor into account.**

**Results are reported as total concentration of cystatin C (ng/ml) in serum/plasma samples.**

For the determination of concentration in samples diluted differently, use dilution factor for dividing/multiplying results read off the standard curve.

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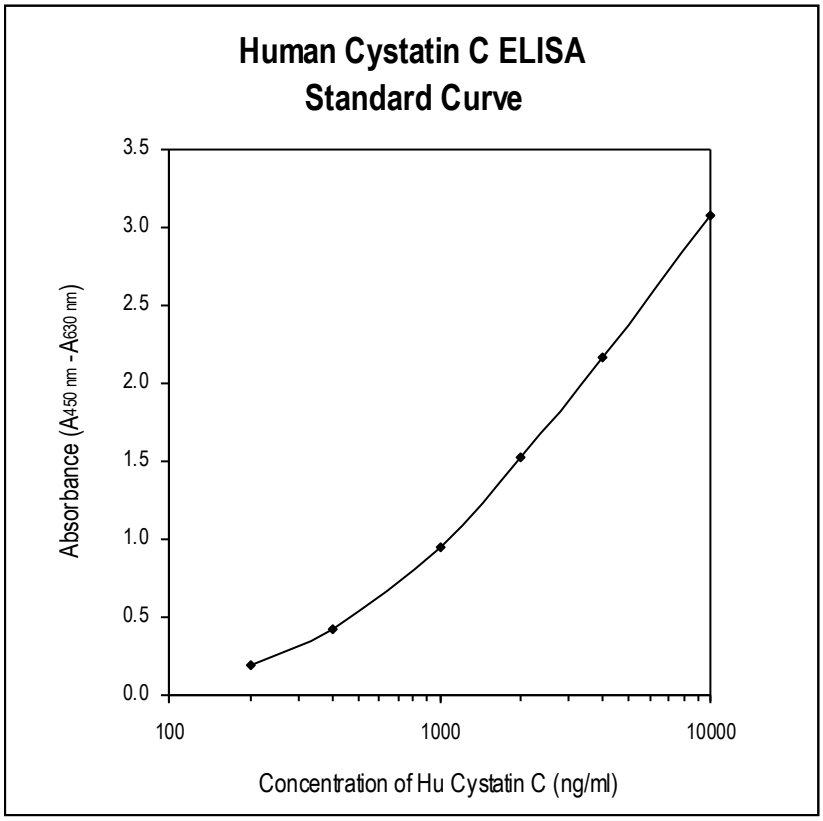


*Figure 2: Typical Standard Curve for Human Cystatin C ELISA.*

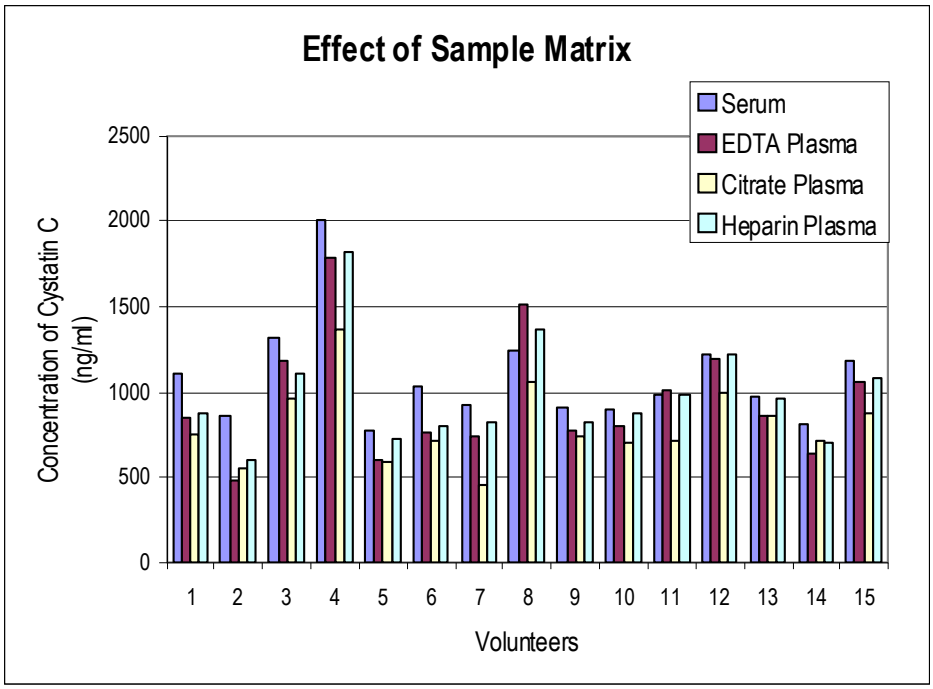


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*Figure. 3:* Cystatin C levels measured using Human cystatin C ELISA from 15 individuals using serum, EDTA, citrate and heparin plasma, respectively

### 12.1 Stability of samples stored at 2-8°C

Samples should be stored at -20°C. However, no decline in concentration of cystatin C was observed in serum and plasma samples after 14 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

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| Sample Number: | Incubation: Temperature, Period | Serum (ng/ml) | Plasma (ng/ml) EDTA | Citrate | Heparin |
|---|---|---|---|---|---|
| 1 | - 20 °C | 1111 | 850 | 751 | 866 |
| | 2 - 8°C, 7 days | 836 | 818 | 668 | 818 |
| | 2 - 8°C, 14 days | 856 | 818 | 751 | 842 |
| 2 | - 20 °C | 861 | 474 | 549 | 600 |
| | 2 - 8°C, 7 days | 839 | 519 | 525 | 585 |
| | 2 - 8°C, 14 days | 701 | 524 | 542 | 528 |
| 3 | - 20 °C | 775 | 606 | 593 | 721 |
| | 2 - 8°C, 7 days | 688 | 652 | 506 | 563 |
| | 2 - 8°C, 14 days | 681 | 707 | 588 | 668 |
| 4 | - 20 °C | 1035 | 760 | 716 | 800 |
| | 2 - 8°C, 7 days | 996 | 743 | 723 | 740 |
| | 2 - 8°C, 14 days | 985 | 731 | 750 | 693 |
| 5 | - 20 °C | 921 | 738 | 459 | 828 |
| | 2 - 8°C, 7 days | 890 | 688 | 545 | 781 |
| | 2 - 8°C, 14 days | 871 | 763 | 560 | 742 |

### 12.2 Effect of Freezing/Thawing

No decline was observed in concentration of human cystatin C in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

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| Sample Number: | Number of f/t Cycles | Serum (ng/ml) | Plasma (ng/ml) | | |
|---|---|---|---|---|---|
| | | | EDTA | Citrate | Heparin |
| 1 | 1x | 1007 | 893 | 666 | 861 |
| | 3x | 1249 | 989 | 660 | 1025 |
| | 5x | 1112 | 966 | 920 | 1063 |
| 2 | 1x | 696 | 683 | 660 | 772 |
| | 3x | 699 | 790 | 660 | 757 |
| | 5x | 667 | 847 | 642 | 768 |
| 3 | 1x | 1329 | 1389 | 1092 | 1528 |
| | 3x | 1367 | 1338 | 1158 | 1550 |
| | 5x | 1344 | 1319 | 1254 | 1447 |
| 4 | 1x | 1970 | 1771 | 1276 | 1891 |
| | 3x | 1930 | 1753 | 1539 | 1963 |
| | 5x | 1979 | 1643 | 1827 | 1994 |
| 5 | 1x | 738 | 715 | 619 | 699 |
| | 3x | 758 | 821 | 552 | 738 |
| | 5x | 649 | 753 | 537 | 844 |

## 13 DEFINITION OF THE STANDARD

The Standard used in this kit is purified native protein based.

## 14 URINE CYSTATIN C DETERMINATION

For the determination of cystatin C in urine use the serum/plasma protocol only with the following modifications:

### 14.1 Sample collection and storage

It is recommended to freeze down untreated urine although no significant decline was observed in concentration of human cystatin C in samples stored at 4°C for 14 days.

### 14.2 Sample preparation

Dilute urine samples **20x** with Dilution Buffer just prior to use in the assay,

e.g.: 20 µl of sample + 380 µl of Dilution Buffer.

*Stability and storage:*

Untreated urine samples are stable for 3 months if stored at -20°C/ -70°C. **Do not store the diluted samples.**

### 14.3 Calculations of results

Standard curve is plotted using values of undiluted Standards: 10 000, 4 000, 2 000, 1 000, 400 and 200 ng/ml.

As urine samples are diluted only **20x** whereas Standards are diluted **400x**, the result (read off the Standard curve) has to be devided by **dilution factor 20** in order to obtain the real concentration in the original (undiluted) sample.

### 14.4 Effect of freezing/thawing on the concentration of cystatin C in urine

Cystatin C levels were determined in the morning urine from fifteen individuals who were examined because of a suspicion of renal dysfunction. All of them had urine protein < 0.3 g/day and a normal count of leukocytes in urine.

Assay results are shown below:

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| Sample No. | Cystatin C (ng/ml) | |
|---|---|---|
| | **1x F/T** | **5x F/T** |
| 1 | 31 | 33 |
| 2 | 62 | 66 |
| 3 | 30 | 22 |
| 4 | 11 | 13 |
| 5 | 24 | 24 |
| 6 | 22 | 24 |
| 7 | 48 | 42 |
| 8 | 32 | 30 |
| 9 | 27 | 32 |
| 10 | 101 | 95 |
| 11 | 39 | 41 |
| 12 | 51 | 63 |
| 13 | 10 | 8 |
| 14 | 84 | 86 |
| 15 | 47 | 43 |

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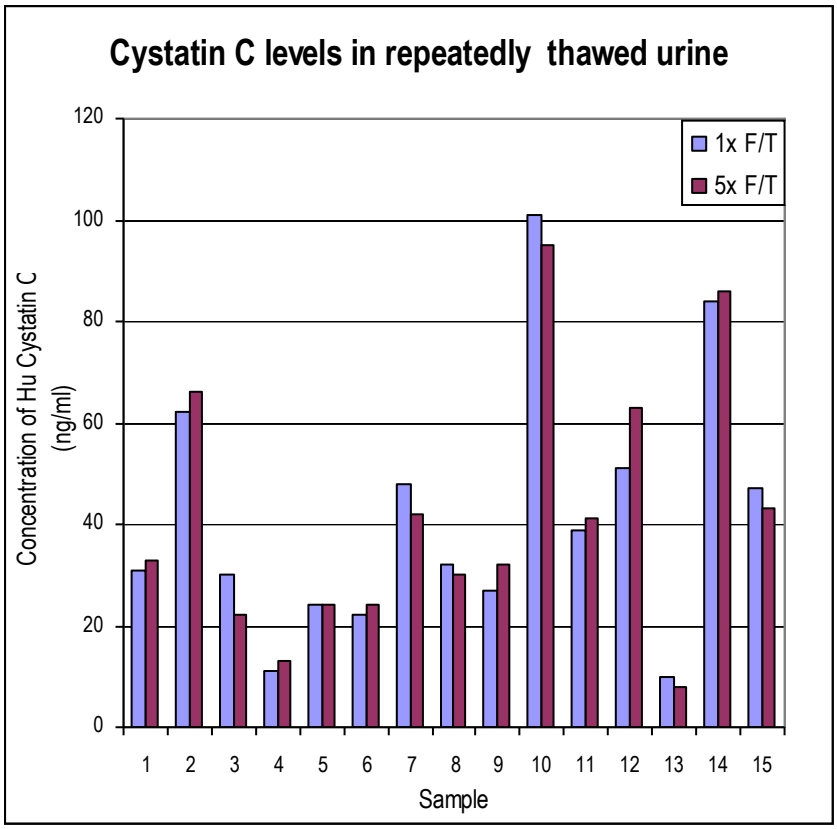


*Figure 4: Cystatin C concentration was determined in urine after repeated freeze-thaw cycles. Samples were taken from fifteen individuals who were suspected to have renal dysfunction.*

## 15 PRELIMINARY POPULATION AND CLINICAL DATA

### 15.1 Serum cystatin C determination

Sera from eight samples on long-term dialysis were measured and their cystatin C levels compared to control sera from ten normal, apparently healthy individuals:

| Sample No. | Cystatin C (ng/ml) | CV (%) |
|---|---|---|
| 1 | 8335 | 6 |
| 2 | 8014 | 8 |
| 3 | 6822 | 1 |
| 4 | 9464 | 8 |
| 5 | 7844 | 8 |
| 6 | 3366 | 4 |
| 7 | 5955 | 1 |
| 8 | 3583 | 14 |

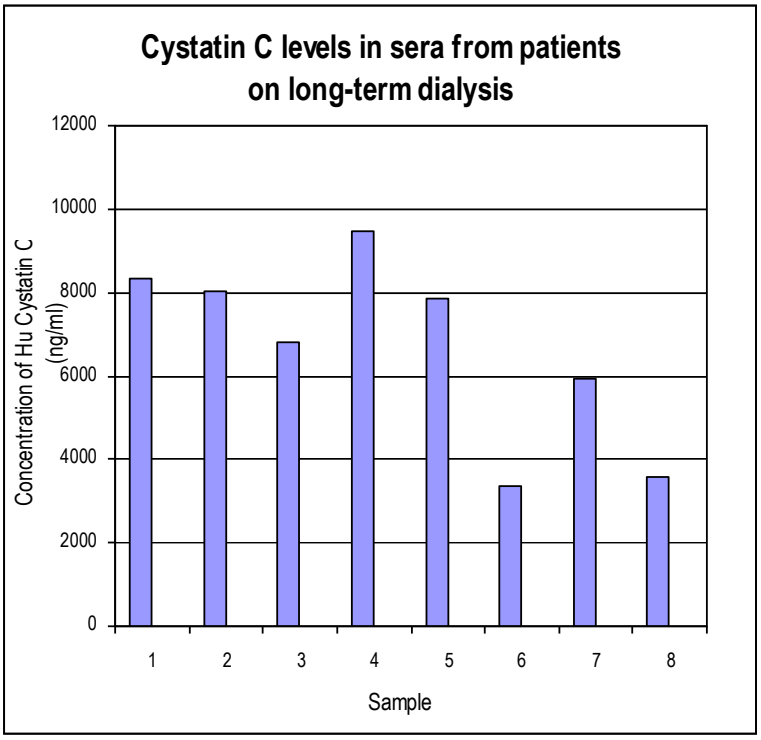

*Figure 5: Cystatin C concentration was determined in serum samples from eight samples on long-term dialysis and compared to control sera.*

| Sample No. | Cystatin C *(ng/ml)* | CV (%) |
|---|---|---|
| pooled serum | 1032 | 11 |
| 1 | 885 | 9 |
| 2 | 979 | 4 |
| 3 | 703 | 8 |
| 4 | 1178 | 6 |
| 5 | 943 | 8 |
| 6 | 751 | 9 |
| 7 | 850 | 5 |
| 8 | 1532 | 6 |
| 9 | 1328 | 2 |

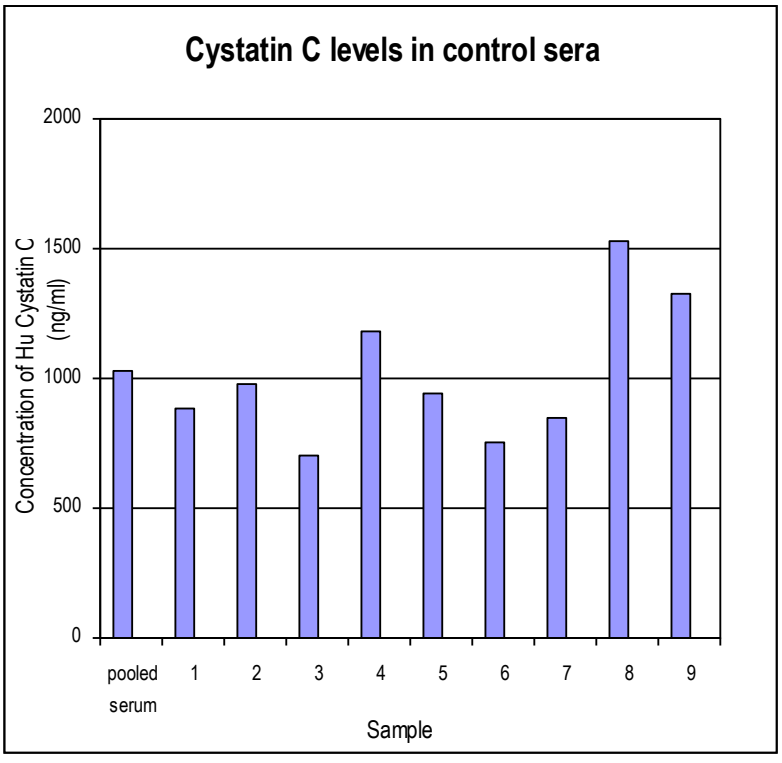


*Figure 6. Samples from nine volunteers and a pooled serum were used as control sera.*

### 15.2 Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological references ranges for cystatin C levels with the assay.

## 16 TROUBLESHOOTING AND FAQS

**Weak signal in all wells**

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

**High signal and background in all wells**

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

**High coefficient of variation (CV)**

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

## 17 REFERENCES / LITERATURE


- Lindahl P, Abrahamson M, Bjork I: Interaction of recombinant human cystatin C with the cysteine proteinases papain and actinidin. *Biochem J.* **281**: 49-55 (1992)
- Ekiel I and Abrahamson M: Folding-related dimerization of human cystatin C. *J Biol Chem*. **271**: 1314-1321 (1996)
- Ekiel I, Abrahamson M, Fulton DB, Lindahl P, Storer AC, Levadoux W, Lafrance M, Labelle S, Pomerleau Y, Groleau D, LeSauteur L, Gehring K: NMR structural studies of human cystatin C dimmers and monomers. *J Mol Biol*. **271**: 266-277 (1997)
- Tian S, Kusano E, Ohara T, Tabei K, Itoh Y, Kawai T, Asano Y: Cystatin C measurement and its practical use in patients with various renal diseases. *Clin Nephrol.* **48**: 104-108 (1997)
- Walker LC: Animal models of cerebral beta-amyloid angiopathy. *Brain Res Rev.* **25**: 70-84 (1997)

- Lerner UH, Johansson L, Ranjso M, Rosenquist JB, Reinholt F., Grubb A: Cystatin C, an inhibitor of bone resorption produced by osteoblasts. *Acta Physiol Scand.* **161**: 81-92 (1997)
- Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J: Cystatin C – a new marker of glomerular filtration rate in children independent of age and height. *Pediatrics.* **101**: 875-881 (1998)
- Shi GP, Sukhova GK, Grubb A, Ducharme A, Rhode LH, Lee RT, Ridker P, Libby P, Chapman HA: Cystatin C deficiency in human atherosclerosis and aortic aneurysms. *J Clin Invest.* **104**: 1191-1197 (1999)
- Risch L, Blumberg A, Huber A: Rapid and accurate assessment of glomerular filtration rate in patients with renal transplants using serum cystatin C. *Nephrol Dial Transplant.* **14**: 1991-1996 (1999)
- Le Bricon T, Thervet E, Benlakehal M, Bousquet B, Legendre C, Erlich D: Changes in plasma cystatin C after renal transplantation and acute rejection in adults. *Clin Chem.* **45**: 2243-2249 (1999)
- Cimerman N, Brguljan PM, Krasovec M, Suskovic S, Kos J: Twenty-four hour variations of cystatin C and total cysteine proteinase inhibitory activity in sera from healthy subjects. *Clin Chim Acta.* **291**: 89-95 (2000)
- Stabuc B, Vrhovec L, Stabuc-Silih M, Cizej TE: Improved prediction of decreased creatinine clearance by serum cystatin C: use in cancer patients before and during chemotherapy. *Clin Chem.* **46**: 193-197 (2000)
- Mangge H, Liebmann P, Tanil H, Herrmann J, Wagner C, Gallistl S, Schauenstein K, Erwa W: Cystatin C, an early indicator for incipient renal disease in rheumatoid arthritis. *Clin Chim Acta.* **300**: 195-202 (2000)
- Takeuchi M, Fukuda Y, Nakano I, Katano Y, Hayakawa T: Evaluation of serum cystatin C concentrations in patients with chronic liver disease. *Eur J Gastroenterol Hepatol.* **13**: 951-955 (2001)
- Bokenkamp A, Dieterich C, Dressler F, Muhlhaus K, Gembruch U, Bald R, Kirschstein M: Fetal serum concentrations of cystatin C and beta2-microglobulin as predictors of postnatal kidney function. *Am J Obstet Gynecol.* **185**: 468-475 (2001)
- Deng A, Irizarry MC, Nitsch RM, Growdon JH, Rebeck GW: Elevation of cystatin C in susceptible neurons in Alzheimer's disease. *Am J Pathol.* **159**: 1061-1068 (2001)
- Demirtaş S, Bozbaş A, Akbay A, Yavuz Y, Karaca L: Diagnostics value of cystatin C for evaluation of hepatorenal syndrome. *Clin Chim Acta.* **311**: 81-89 (2001)
- Lindholt JS, Erlandsen EJ, Henneberg EW: Cystatin C deficiency is associated with the progression of small abdominal aortic aneurysms. *Br J Surg.* **88**: 1472-1475 (2001)
- Tan GD, Lewis AV, JamesTJ, Altmann P, Taylor RP, Levy JC: Clinical usefulness of cystatin C for the estimation of glomerular filtration rate in type 1 diabetes: reproducibility and accuracy compared with standard measures and iohexol clearance. *Diabetes Care.* **25**: 2004-2009 (2002)
- Mussap M, Dalla Vestra M, Fioretto P, Saller A, Varagnolo M, Nosadini R, Plebani M: Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. *Kidney Int.* **61**: 1453-1461 (2002)
- Kazama JJ, Kutsuwada K, Ataka K, Maruyama H, Gejyo F: Serum cystatin C reliably detects renal dysfunction in patients with various renal diseases. *Nephron.* **91**: 13-20 (2002)
- Uchida K and Gotoh A: Measurement of cystatin-C and creatinine in urine. *Clin Chim Acta.* **323**: 121-128 (2002)
- Dharnidharka VR, Kwon C, Stevens G: Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis.* **40**: 221-226 (2002)
- Mares J, Stejskal D, Vavrouskova J, Urbanek K, Herzig R, Hlustik P: Use of cystatin C determination in clinical diagnostics. *Biomed Papers.* **147**: 177-180 (2003)
- Mojiminiyi OA and Abdella N: Evaluation of cystatin C and beta-2 microglobulin as markers of renal function in patients with type 2 diabetes mellitus. *J Diabetes Complications.* **17**: 160-168 (2003)

- Benussi L, Ghidoni R, Steinhoff T, Alberici A, Villa A, Mazzoli F, Nicosia F, Barbiero L, Broglio L, Feudatari E, Signorini S, Finckh U, Nitsch RM, Binetti G: Alzheimer disease-associated cystatin C variant undergoes impaired secretion. *Neurobiol Dis.* **13:** 15-21 (2003)
- Perlemoine C, Beauvieux MC, Rigalleau V, Baillet L, Barthes N, Derache P, Gin H: Interest of cystatin C in screening diabetic patients for early impairment of renal function. *Metabolism.* **52**: 1258-1264 (2003)
- Wiesli P, Schwegler B, Spinas GA, Schmid C: Serum cystatin C is sensitive to small changes in thyroid function. *Clin Chim Acta.* **338**: 87-90 (2003)
- Watanabe S, Okura T, Liu J, Miyoshi K, Fukuoka T, Hiwada K, Higaki J: Serum cystatin C level is a marker of end-organ damage in patients with essential hypertension. *Hypertens Res.* **26**: 895-899 (2003)
- Mussap M and Plebani M: Biochemistry and Clinical Role of Human Cystatin C. *Crit Rev Clin Lab Sci.* **41**: 467-550 (2004)
- Johnston N, Jernberg T, Lindahl B, Lindback J, Stridsberg M, Larsson A, Venge P, Wallentin L: Biochemical indicators of cardiac and renal function in a healthy elderly population. *Clin Biochem.* **37**: 210-216 (2004)
- Weiner DE, Tighiouart H, Stark PC, Amin MG, MacLeod B, Griffith JL, Salem DN, Levevy AS, Sarnak MJ: Kidney disease as a risk factor for recurrent cardiovascular disease and mortatlity. *Am J Kidney Dis.* **44**: 198-206 (2004)
- Jernberg T, Lindhal B, James S, Larsson A, Hansson LO, Wallentin L: Cystatin C: a novel predictor of outcome in suspected or confirmed non-ST-elevation acute coronary syndrome. *Circulation.* **110**: 2342-2348 (2004)
- Koenig W, Twardella D, Brenner H, Rothenbacher D: Plasma Concentrations of Cystatin C in Patients with Coronary Heart Disease and Risk for Secondary Cardiovascular Events: More Than Simply a Marker of Glomerular Filtration Rate. *Clin Chem.* (2004)
- Shlipak MG, Sarnak MJ, Katz R, Fried L, Seliger S, Newman A, Siscovick D, Stehman-Breen C: Cystatin-C and mortality in elderly persons with heart failure. *J Am Coll Cardiol.* **45**: 268-271 (2005)
- Artunc FH, Fischer IU, Risler T, Erley CM: Improved estimation of GFR by serum cystatin C in patients undergoing cardiac catheterization. *Int J Cardiol.* **102**: 173-178 (2005)
- Luc G, Bard JM, Lesueur C, Arveiler D, Evans A, Amouyel P, Ferrieres J, Juhan-Vague I, Fruchart JC, Ducimetiere P: Plasma cystatin-C and development of coronary heart disease: The PRIME Study. *Atherosclerosis.* (2005)
- Noto D, Cefalu AB, Barbagallo CM, Pace A, Rizzo M, Marino G, Caldarella R, Castello A, Pernice V, Notarbartolo A, Averna MR: Cystatin C levels are decreased in acute myocardial infarction; Effect of cystatin C G73A gene polymorphism on plasma levels. *Int J Cardiol.* **101**: 213-217 (2005)
- Conti M, Zater M, Lallali K, Durrbach A, Moutereau S, Manivet P, Eschwege P, Loric S: Absence of circadian variations in urine cystatin C allows its use on urinary samples. *Clin Chem.* **51**: 272-273 (2005)
- Seliger SL, Longstreth WT Jr., Kratz R, Manolio T, Fried LF, Shlipak M, Stehman-Breen CO, Newman A, Sarnak M, Gillen DL, Bleyer A, Siscovick DS: Cystatin C and subclinical brain infarction. *J Am Soc Nephrol*. **16**: 3721-3727 (2005)
- Fried LF, Kratz R, Sarnak MJ, Shlipak MG, Chaves PH, Jenny NS, Stehman-Breen C, Gillen D, Bleyer AJ, Hirsch C, Siscovick D, Newman AB: Kidney function as a predictor of noncardiovascular mortality. *J Am Soc Nephrol.* **16**: 3728-3735 (2005)
- Shlipak MG, Wassel Fyr CL, Chertow GM, Harris TB, Kritchevsky SB, Tylavsky FA, Satterfield S, Cummings SR, Newman AB, Fried LF: Cystatin C and mortality risk in the elderly: the health, aging, and body composition study. *J Am Soc Nephrol.* **17**: 254-261 (2006)

- Kato T, Heike T, Okawa K, Haruyama M, Shiraishi K, Yoshimoto M, Nagato M, Shibata M, Kumada T, Yamanaka Y, Hatorri H, Nakahata T: A neurosphere-derived factor, cystatin C, supports differentiation of ES cells into neural stem cells. *Proc Natl Acad Sci U S A*. **103**: 6019-6024 (2006)
- Conti M, Moutereau S, Zater M, Lallali K, Durrbach A, Manivet P, Eschwege P, Loric S: Urinary cystatin C as a specific marker of tubular dysfunction. *Clin Chem Lab Med.* **44**: 288-291 (2006)
- Macisaac RJ, Tsalamandris C, Thomas MC, Premaratne E, Panagiotopoulos S, Smith TJ, Poon A, Jenkins MA, Ratnaike SI, Power DA Jerums G: Estimating glomerular filtration rate in diabetes: a comparison of cystatin-C- and creatinine-based methods. *Diabetologia.* **49**: 1686-1689 (2006)
- Nakashima I, Fujihara K, Fujinoki M, Kawamura T, Nishimura T, Nakamura M, Itoyama Y: Alteration of cystatin C in the cerebrospinal fluid of multiple sclerosis. *Ann Neurol.* (2006)
- Fried LF, Biggs ML, Shlipak MG, Seliger S, Kestenbaum B, Stehman-Breen C, Sarnak M, Siscovick D, Harris T, Cauley J, Newman AB, Robbins J: Association of kidney function with incident hip fracture in older adults. *J Am Soc Nephrol*. **18**: 282-286 (2007)
- Delanaye P, Cavalier E, Krzesinski JM.: Cystatin C, renal function, and cardiovascular risk. *Ann Intern Med.* Jul **3;**147(1):19-27 (2007)
- Herget-Rosenthal S, van Wijk JA, Bröcker-Preuss M, Bökenkamp A.: Increased urinary cystatin C reflects structural and functional renal tubular impairment independent of glomerular filtration rate. *Clin Biochem.* Sep;**40**(13-14):946-51(2007)
- Delanaye P, Cavalier E, Radermecker RP, Paquot N, Depas G, Chapelle JP, Scheen AJ, Krzesinski JM: Cystatin C or creatinine for detection of stage 3 chronic kidney disease in anorexia nervosa. *Nephron Clin Pract.* **110**(3):c158-63 (2008)
- Parikh NI, Hwang SJ, Yang Q, Larson MG, Guo CY, Robins SJ, Sutherland P, Benjamin EJ, Levy D, Fox CS: Clinical correlates and heritability of cystatin C (from the Framingham Offspring Study). *Am J Cardiol.* Nov 1;**102**(9):1194-8 (2008)
- Sundelöf J, Arnlöv J, Ingelsson E, Sundström J, Basu S, Zethelius B, Larsson A, Irizarry MC, Giedraitis V, Rönnemaa E, Degerman-Gunnarsson M, Hyman BT, Basun H, Kilander L, Lannfelt L: Serum cystatin C and the risk of Alzheimer disease in elderly men. *Neurology* Sep 30;**71**(14):1072-9 (2008)
- Ortiz F, Harmoinen A, Paavonen T, Koskinen P, Grönhagen-Riska C, Honkanen E: Is Cystatin C more sensitive than creatinine in detecting early chronic allograft nephropathy? *Clin Nephrol.* **70**(1):18-25 (2008)
- Samouilidou EC, Grapsa E.: Relationship of serum cystatin C with C-reactive protein and apolipoprotein A1 in patients on hemodialysis. *Ren Fail.* **30**(7):711-5(2008)
- Muntner P, Mann D, Winston J, Bansilal S, Farkouh ME.: Serum cystatin C and increased coronary heart disease prevalence in US adults without chronic kidney disease. *Am J Cardiol.* Jul **1**;102(1):54-7 (2008)
- Servais A, Giral P, Bernard M, Bruckert E, Deray G, Isnard Bagnis C.: Is serum cystatin-C a reliable marker for metabolic syndrome? *Am J Med.* May;**121**(5):426-32 (2008)
- Muntner P, Winston J, Uribarri J, Mann D, Fox CS.: Overweight, obesity, and elevated serum cystatin C levels in adults in the United States. *Am J Med.* Apr;**121**(4):341-8 (2008)
- Premaratne E, MacIsaac RJ, Finch S, Panagiotopoulos S, Ekinci E, Jerums G.: Serial measurements of cystatin C are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes. *Diabetes Care.* May;**31**(5):971-3 (2008).
- Rehman T, Fought J, Solomon R.: N-acetylcysteine effect on serum creatinine and cystatin C levels in CKD patients. *Clin J Am Soc Nephrol.* Nov;**3**(6):1610-4 (2008)

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- Moran A, Katz R, Smith NL, Fried LF, Sarnak MJ, Seliger SL, Psaty B, Siscovick DS, Gottdiener JS, Shlipak MG.: Cystatin C concentration as a predictor of systolic and diastolic heart failure. *J Card Fail.* Feb;**14**(1):19-26 (2008)
- Hossain M. A., Emara M., El moselhi. H., Shoker A.: Comparing measures of Cystatin C in human sera by three methods. *Am J Nephrology* **29(5):** 381 – 391 (2009)
- Nedelkov D and Nelson RW: Delineating protein-protein interactions via biomolecular interaction analysis-mass spectrometry. *J Mol Recognit.* **16**: 9-14 (2003)
- Nedelkov D and Nelson RW: Design and use of multi-affinity surfaces in biomolecular interaction analysis-mass spectrometry (BIA/MS): a step toward the design of SPR/MS arrays. *J Mol Recognit.* **16**: 15-19 (2003)
- Straface E, Matarrese P, Gambardella L, Vona R, Sgadari A, Silveri MC, Malorni W: Oxidative imbalance and cathepsin D changes as peripheral blood biomarkers of Alzheimer disease: A pilot study. *FEBS Lett*. **579**: 2759-2766 (2005)
- Stejskal D, Vavrouskova J, Mares J, Urbanek K: Applications of new laboratory marker assays in neurological diagnoses - A pilot study. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*. **149**: 265-266 (2005)
- Taleb S, Lacasa D, Bastard JP, Poitou C, Cancello R, Pelloux V, Viguerie N, Benis A, Zucker JD, Bouillot JL, Coussieu C, Basdevant A, Langin D, Clement K: Cathepsin S, a novel biomarker of adiposity: relevance to atherogenesis. *FASEB J.* **19**: 1540-1542 (2005)
- Barrientos LG, Rollin PE: Release of cellular proteases into the acidic extracellular milieu exacerbates Ebola virus-induced cell damage. *Virology* (2006)
- Liu XD, Zeng BF, Xu JG, Zhu HB, Xia QC: Proteomic analysis of the cerebrospinal fluid of patients with lumbar disk herniation. *Proteomics* **6**: 1019-1028 (2006)
- Taleb S, Cancello R, Clement K, Lacasa D: Cathepsin S promotes human preadipocyte differentiation: possible involvement of fibronectin degradation. *Endocrinology*. **147**: 4950-4959 (2006)
- Yang X, Wang H, Wang Z, Dong M: Alteration and significance of serum cardiac troponin I and cystatin C in preeclampsia. *Clin Chim Acta.* **374**: 168-169 (2006)
- Taleb S, Cancello R, Poitou C, Rouault C, Sellam P, Levy P, Bouillot JL, Coussieu C, Basdevant A, Guerre-Millo M, Lacasa D, Clement K: Weight loss reduces adipose tissue cathepsin S and its circulating levels in morbidly obese women. *J Clin Endocrinol Metab.* **91**: 1042-1047 (2006)

## 18 PLATE LAYOUT

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