This kit employs a sandwich enzyme immunoassay technique to quantify cystatin C in biological samples.

Human cystatin C ELISA Kit
Sandwich ELISA
Catalog EL-213-03

INTRODUCTION

Cystatin C is a small, roughly 13 kDa secreted protein expressed constitutively by nearly every nucleated cell in the body and released into blood, cerebrospinal fluid, and other body fluids at high, stable concentrations. Its core biochemical role is inhibiting cysteine proteases, including lysosomal cathepsins, which lets it act as a local brake on proteolytic activity in tissues and also gives it some antimicrobial activity by restricting viral replication. Because it is filtered and cleared by the kidney at a fairly constant rate regardless of muscle mass, diet, or sex, cystatin C has become a widely used clinical marker for estimating glomerular filtration rate, often as a complement or alternative to creatinine. Beyond the kidney, altered cystatin C biology is tied to vascular disease -- reduced expression is seen in smooth muscle of atherosclerotic and aneurysmal vessel walls -- and rare mutations cause a hereditary form of cerebral amyloid angiopathy in which the protein misfolds and accumulates in blood vessel walls, leading to intracranial hemorrhage.

PRINCIPLE OF THE ASSAY

This kit uses a sandwich enzyme immunoassay format. The microtiter plate is pre-coated with a capture antibody specific to human cystatin C, which immobilizes the target analyte from standards or samples added to each well. An HRP-conjugated detection antibody, also specific to human cystatin C, is then added and binds a second epitope on the captured analyte. Following the addition of TMB substrate solution, wells containing the cystatin C–HRP-antibody complex develop a colored product. The enzymatic reaction is stopped by the addition of sulfuric acid stop solution, and absorbance is measured at 450 ± 10 nm. Sample cystatin C concentrations are determined by comparing well absorbance values against a standard curve.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard Microplate	2 strips x 8 wells
HRP Conjugate Antibody (100×)	70 µL
Standard/Sample Diluent Buffer	24 mL
HRP Conjugate Diluent	10 mL
Wash Buffer (25×)	24 mL
TMB Substrate Solution	12 mL
Stop Reagent	7 mL
Plate Covers	2 pieces
Instruction Manual	1
Do not mix or substitute reagents with those from other lots.	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient

sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3’,5,5’-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
2. **Detection Reagent (1X) Preparation: Dilute the HRP-conjugated detection antibody concentrate 100-fold with HRP Conjugate Diluent, immediately before use.**
3. **Preparation of Calibrators: Prepare calibrators by serial dilution from a 5000 down to 78.13 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (µL)	Cal. Diluent (µL)	Final Vol (µL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	5000
Std 2	Std 1	500	500	1000	2500
Std 3	Std 2	500	500	1000	1250
Std 4	Std 3	500	500	1000	625
Std 5	Std 4	500	500	1000	312.5
Std 6	Std 5	500	500	1000	156.25
Std 7	Std 6	500	500	1000	78.13
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1.After the kit is equilibrated at room temperature, add 50µL Standard/Sample Diluent Buffer to each Standard well , and add 50 µL sample to the sample well, Immediately add 50 µL 1× HRP Conjugate Antibody Working Solution to each well, Incubate at 37°C on Microplate . oscillator for 60 min

2.Discard the liquid in the plate, add 200 µL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 µL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.

3.Add 50 µL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
2. The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 25 pg/mL

Recovery: Serum (n = 5): 88-99% (avg 94%); EDTA plasma (n = 5): 102-110%

(avg 104%); Heparin plasma (n = 5): 98-104% (avg 101%)

Specificity: This assay has high sensitivity and excellent specificity for detection of Human Cys-C. No significant cross-reactivity or interference between Human Cys-C and analogues was observed.

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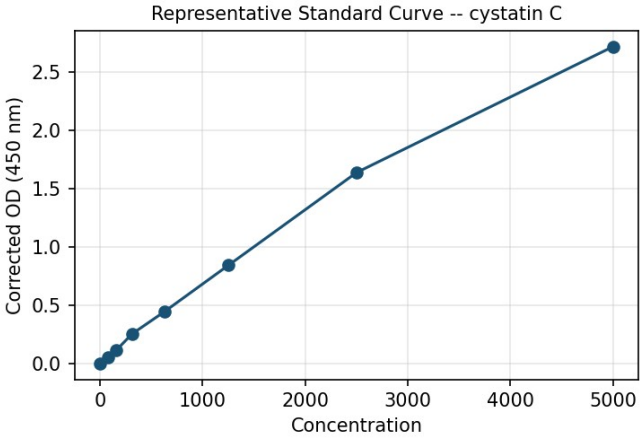
SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit *www.affinityimmuno.com* to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.