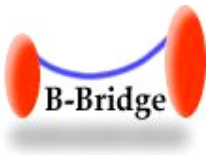

Human Cystatin C
Enzyme Immunoassay Kit

User Manual

Catalog# K3012-1

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Notice to Purchaser

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INTENDED USE

The B-Bridge Human Cystatin C ELISA kit (catalog # K3012-1) is designed to quantitatively measure human Cystatin C present in serum, EDTA and Heparin plasma, urine, and tissue culture media; samples containing visible particulate should be centrifuged prior to using. This assay has been shown to detect Cystatin C from human samples only. Please read the complete kit insert before performing this assay.

BACKGROUND

Cystatin C is a non-glycosylated protein of low molecular weight (13kDa) in the cystatin superfamily. Cystatin C is produced at a constant rate in all nucleated cells, secreted from cells and thus found in detectable amounts in most body fluids. Cystatin C belongs to the cysteine proteinase inhibitor group and is associated with several pathological conditions. Imbalance between Cystatin C and cysteine proteases is associated with diseases such as inflammation, renal failure, cancer, Alzheimer's disease, multiple sclerosis and hereditary Cystatin C amyloid angiopathy. Increased levels have been found in autoimmune diseases, with colorectal tumors and metastases, patients with inflammation and in patients on dialysis. Cystatin C is removed from blood plasma by glomerular filtration in the kidneys. It is reabsorbed by the proximal tubular cells and degraded. There is a linear relationship between the reciprocal Cystatin C concentration in plasma and the glomerular filtration rate (GFR). Cystatin C is suggested to be a better marker for GFR than the ubiquitous serum creatinine marker as its serum concentration is not affected by other factors such as age, gender and body mass and Cystatin C has higher sensitivity to detect a reduced GFR than creatinine determination. Low levels of Cystatin C are found with the breakdown of the elastic laminae and atherosclerosis and abdominal aortic aneurysm. There is evident association of Cystatin C levels with the incidence of myocardial infarction, coronary death and angina pectoris, presenting a risk factor for secondary cardiovascular events.

ASSAY PRINCIPLE

The B-Bridge Human Cystatin C ELISA Kit is designed to measure Cystatin C present in human biological samples and tissue culture media. Standards or diluted samples are pipetted into a clear microtiter plate coated with a mouse anti-human Cystatin C monoclonal antibody. After a 60 minute incubation, the plate is washed and a peroxidase conjugated Cystatin C monoclonal antibody is added. After incubation and washing, substrate is added to the plate. The plate is read at 450 nm wavelength and concentration of Cystatin C in the sample is calculated using software available with most plate readers.

KIT COMPONENTS

Clear Coated 96-well plate	One Plate
Cystatin C Standard (400ng/mL)	60 uL
Cystatin C Conjugate	5 mL
5X Assay Buffer Concentrate	28 mL
20X Wash Buffer Concentrate	30 mL
TMB Substrate	5 mL
Stop Solution - 1N hydrochloric acid solution (HCl). **CAUSTIC**	5 mL
Plate Sealer	1 each

Store above components at 4°C

MATERIALS REQUIRED BUT NOT SUPPLIED

- Supply of distilled or deionized water
- Microplate washer
- Colorimetric 96-well microplate reader capable of reading OD at 450 nm with correction between 570 and 590 nm.
- Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting

PRECAUTIONS

For in vitro research use only. Read and understand the complete user manual before attempting to use the product. As with all such products, this kit should only be used by qualified personnel who are experienced in routine laboratory procedures and safety practices.

The Cystatin C Standard is purified from a human source and should be treated as potentially hazardous. Proper safety procedures must be followed. **The Stop Solution is 1N hydrochloric acid. Appropriate precautions should be observed when handling this caustic solution. Avoid contact with skin or eyes.**

Wear gloves and laboratory coats when handling materials and, in all cases, please consult your institution's safety procedures for working with hazardous chemicals.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes.

We recommend that all standards and samples be run in for accurate determination of Cystatin C concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer
Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer
Dilute 20X Wash Buffer 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Standard Preparation
Using the Cystatin C stock at (400 ng/mL), prepare a series of 7 standards by serial dilution as follows, referring to the below table. Label seven glass test tubes as #1 through #7. Briefly spin vial of standard in a microcentrifuge to ensure contents are at the bottom of the vial.

- Pipet 585 µL of Assay Buffer into tube 1 and 250 µL into tubes 2 to 7.
- Add 15 µL of the Cystatin C stock solution to tube 1 and vortex completely.
- Take 250 µL of the Cystatin C solution in tube 1 and add it to tube 2 and vortex completely. Repeat the serial dilutions for tubes 3 through 7.
- The concentration of Cystatin C in tubes 1 through 7 will be 10, 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/mL.

Use all Standards within 2 hours of preparation.

Reagent	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5	Standard 6	Standard 7
Assay Buffer Volume	585 µl	250 µl	250 µl	250 µl	250 µl	250 µl	250 µl
Cystatin C Stock	15 µl						
Standard 1		250 µl					
Standard 2			250 µl				
Standard 3				250 µl			
Standard 4					250 µl		
Standard 5						250 µl	
Standard 6							250 µl
Final Concentration (ng/mL)	10	5	2.5	1.25	0.625	0.313	0.156

SAMPLE PREPARATION

This assay has been validated for human serum, EDTA and heparin plasma, urine and tissue culture media samples. Samples containing visible particulate should be centrifuged prior to using. This assay has been shown to detect Cystatin C from only human samples.

Use samples within 2 hours of dilution.

Serum and Plasma Samples
Serum and plasma samples must be diluted at least 1:50 with the provided Assay Buffer immediately prior to use. A dilution of at least 1:225 is recommended to detect most samples within the standard curve range. Disease state samples (e.g. sample from tubular kidney disease) may require further dilutions up to 1:500 or greater. Appropriate sample dilutions should be determined empirically.

Urine Samples
Urine samples must be diluted at least 1:4 with the provided Assay Buffer prior to use with the kit.

Tissue Culture Media
Tissue culture medium samples should be diluted in tissue culture medium and read off a standard curve generated in the same culture medium.

ASSAY PROTOCOL

Standards and samples should be run in duplicate

1. Pipet 50 µL of sample or standard into each well. Pipet 50 µL of Assay Buffer into the zero standard wells.
2. Incubate at room temperature for 60 minutes. Aspirate the plate and wash 4 times with the wash buffer. Tap the plate dry on absorbent towels.
3. Add 50 µL of the Cystatin C Conjugate to each well, using a repeater or multi-channel pipet.
4. Incubate at room temperature for 30 minutes
5. Aspirate the plate and wash 4 times with the wash buffer. Tap the plate dry on absorbent towels.
6. Add 50 µL of the TMB Substrate to each well, using a repeater or a multichannel pipet.
7. Incubate at room temperature for 30 minutes.
8. Add 50 µL of the Stop Solution to each well.
9. Read the optical density at wavelengths 450 nm with correction between 570 and 590 nm.

CALCULATIONS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

TYPICAL STANDARD CURVE: EXAMPLE ONLY

Standard curves vary with each assay. Always run your own standard curves for calculation of results; do not use this data.