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Product Manual

Cystatin C ELISA Kit

Catalog Number：84601

96 assays

NewEast Biosciences
24 Whitewoods Lane
Malvern, PA 19355

Fax: 610-945-2008
Phone: 610-945-2007
Web: www.neweastbio.com
E-mail: sale@neweastbio.com

Table of Content

Product Description	**3**
Assay Principle	**3**
Kit Components	**3**
Storage	**4**
Reagent Preparation	**4**
Sample Preparation	**5**
Assay Procedure	**7**
Calculation of Results	**8**
Typical Standard Curves	**9**
Sensitivity	**9**

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Product Description

Cystatin C, a protein encoded by the CST3 gene, is mainly used as a biomarker of kidney function. Cystatin C has a low molecular weight (approximately 13.3 kilodaltons), and it is removed from the bloodstream by glomerular filtration in the kidney. If kidney function and glomerular filtration rate decline, the blood levels of cystatin C rise. Serum levels of cystatin C are a more precise test of kidney function (as represented by the glomerular filtration rate, GFR) than serum creatinine levels. Cystatin C levels are less dependent on age, sex, race and muscle mass compared to creatinine.

NewEast Biosciences Cystatin C ELISA Kit is a simple and fast sandwich enzyme immunoassay (EIA) for in vitro quantitative detection of Cystatin C proteins in cell lysates, blood and urine samples. Each kit provides sufficient quantities to perform 96 assays.

Assay Principle

With the NewEast Biosciences Cystatin C sandwich ELISA assay system, the polyclonal anti-human Cystatin C antibody is pre-coated onto a 96-well plate and is used to capture Cystatin C proteins from a sample. Captured Cystatin C proteins are detected using biotin-labelled monoclonal anti-human Cystatin C antibody and streptavidin-poly-HRP conjugate. After addition of the substrate solution, the amount of Cystatin C is determined. The standard curve demonstrates a direct relationship between Optical Density (OD) and Cystatin C concentration: i.e., the higher the OD the higher the Cystatin C concentration in the sample.

Kit Components

1. Immunoplate Pre-coated with polyclonal anti-human Cystatin C antibody (Catalog No.21108): One Plate of 96 Wells. A plate using break-apart strips coated with polyclonal anti-human Cystatin C antibody.

2. Assay/Diluent Buffer (Catalog No. 30403): One bottle of 30 mL of 100 mM phosphate buffer, pH 7.2, 1% BSA, 0.1% Tween-20 and 0.02% Thimerosol.

3. Biotin-labelled monoclonal anti-human Cystatin C antibody (Catalog No. 26044): One vial of 10 µL (200 µg/ml) in PBS, pH 7.4 (1000 X stock solution and diluent buffer are provided).

3. Streptavidin-poly-HRP conjugate (Catalog No. 29030): One vial of 10 µl (200 µg/ml) in PBS, pH 7.4, containing 50% glycerol (5000 X stock solution and diluent buffer are provided).

4. 10X Wash Buffer Concentrate (Catalog No. 30106): One bottle of 15 mL phosphate buffered saline containing detergents.

5. Cystatin C Standard (Catalog No. 10157): One vial (15 µg in 150 µl) of human Cystatin C protein standard.

6. Substrate A (Catalog No. 30107): One vial 6 mL.

7. Substrate B (Catalog No. 30108): One vial 6 mL.

8. Stop Solution (Catalog No. 30110): One vial 12 mL.
   A solution of oxalic acid in water. Keep tightly capped.

Storage

Store all kit components at 4°C until their expiration dates.
Note: For long-term best results, store biotin-labelled monoclonal anti-human cystatin C antibody (Catalog No. 26044), cystatin C standard (Catalog No. 10157) at -20℃upon receipt. Add 1 µL biotin-labelled monoclonal anti-human cystatin C antibody into 1 mL diluent buffer and mix the solution gently before use. Please avoid repeated thawing and freezing after mixing.

Materials Needed but Not Supplied

1. Multi-channel or repeating pipettes
2. Pipettors & tips capable of accurately measuring 10-1000 µL
3. Graduated serological pipettes
4. 96-well microplate reader capable of measuring absorbance at 405 nm
5. Graph paper for manual plotting of data
6. 1.5 mL tubes
7. Mechanical vortex
8. Two1 liter containers
9. Plate shaker (optional)
10. Distilled or deionized water

Reagent Preparation

1. Cystatin C Standard

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Prepare a set of standards using Dilution Buffer as follows: 0.625 ug/ml, 0.313 ug/ml, 0.156 ug/ml, 0.0781 ug/ml, 0.0391 ug/ml, 0.0195 ug/ml.
Diluted standards should be used within 30 minutes of preparation.

2. Biotin-labelled monoclonal anti-human cystatin C antibody
Immediately before use, dilute the biotin-labelled monoclonal anti-human cystatin C antibody 1:1000 with assay/diluent buffer as follows: For each 20 well strip, prepare 2 mL of diluted biotin labelled monoclonal anti-human cystatin C antibody by adding 2 µL of biotin labelled monoclonal anti-human cystatin C antibody to 1998 µL of assay/diluent buffer.

3. Streptavidin-poly-HRP conjugate
Immediately before use, dilute the Streptavidin-poly-HRP conjugate 1:5,000 with ELISA Assay/Diluent buffer as follows: For each 30 well strip prepare 3 mL of diluted Streptavidin-poly-HRP by adding 0.6 µL of Streptavidin-poly-HRP to 2999.4 µL of Assay/Diluent buffer.

4. Wash Buffer
Prepare the Wash Buffer by diluting 15 mL of the supplied concentrate with 135 mL of deionized water. This can be stored at room temperature until the kit expiration date.

Sample Preparation

Adherent Cells

1. Culture cells (one 10-cm plate, ~ 10^7 cells) to approximately 80-90% confluence. Stimulate cells with activator or inhibitor as desired.
2. Aspirate the culture media and wash twice with ice-cold PBS.
3. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cells (0.5 - 1 mL per 10 cm tissue culture plate).
4. Place the culture plates on ice for 10-20 minutes.
5. Detach the cells from the plates by scraping with a cell scraper.
6. Transfer the lysates to appropriate size tubes and place on ice.
7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
8. Clear the lysates by centrifugation for 10 minutes (12,000 x g at 4 °C).

9. Collect the supernatant and store samples (~1-2 mg of total proteins) on ice for immediate use, or snap freeze and store at - 70 °C for future use.

Suspension Cells

1. Culture cells and stimulate with activator or inhibitor as desired.
2. Perform a cell count, and then pellet the cells by centrifugation.
3. Aspirate the culture media and wash twice with ice-cold PBS.
4. Completely remove the final PBS wash and add ice-cold 1X Assay/Lysis Buffer to the cell pellet (0.5 – 1 mL per 1 x 10^7cells).
5. Lyse the cells by repeated pipetting.
6. Transfer the lysates to appropriate size tubes and place on ice.
7. If nuclear lysis occurs, the cell lysates may become very viscous and difficult to pipette. If this occurs, lysates can be passed through a 27½-gauge syringe needle 3-4 times to shear the genomic DNA.
8. Clear the lysates by centrifugation for 10 minutes (12,000 x g at 4 °C).
9. Collect the supernatant and store samples on ice for immediate use, or snap freeze and store at - 70 °C for future use.

Blood Samples

1. Blood can be assayed directly, but for most assay purposes, it is either allowed to clot or is centrifuged down to pellet out red and white cells.

2. Clotting requires several hours at room temperature, and the clear yellow liquid, the serum, can be used for fascin analysis. More rapid and convenient is to spin the blood down at top speed in an Eppendorf centrifuge and take the clear yellow liquid, the plasma, for analysis. Workable results have been obtained with blood, serum and plasma, but the kit has been standardized on plasma for reproducibility.

3. A series of blood samples can be taken and frozen at -20°C or lower. Then, when a complete series of samples have been collected they can be thawed out and the red and white cells can be pelleted out in microfuge tubes at top speed for 5 minutes in a microfuge centrifuge. The plasma is then run in the ELISA.

Assay Procedure

1. Place the desired number of strips in the strip well plate holder. Return unused strips to the foil pouch. Tape may assist in holding the wells in place during the assay.

2. Add 100 µL of standards or samples to wells (see reagent preparation section). It is recommended that standards and samples be run in duplicate.

Note: A standard curve must be run at each setting.

3. Seal the plate with a plate sealer. Incubate the plate for 40 min at 37℃ with gentle shaking.

4. **IMPORTANT WASH STEP:**

Gently remove the plate sealer and wash the plate at least 3 times. A thorough washing of the plate is extremely important to reduce background. We recommend using a multi-channel pipette to fill each well with 200 µL of diluted Wash Buffer. Fluid removal from the wells is best accomplished by inverting the plate over a sink and flicking the fluid out of the wells and then blotting the plate on clean paper towels. Repeat this procedure for a total of 3 times.

5. Add 100 µL of the diluted biotin-labelled monoclonal anti-human cystatin C antibody (see reagent preparation section) to each well. Cover the plate and incubate the plate for 40 min at 37℃ with gentle shaking.

6. Wash as described in Step 4.

7. Add 100 µL of the diluted Streptavidin-poly-HRP conjugate (see reagent preparation section) to each well. Cover the plate and incubate the plate for 15 min at 37℃ with gentle shaking.

8. Wash as described in Step 4.

9. Add 100 µL of the Substrate solution to every well. Incubate at room temperature for 5~30 minutes without shaking. (**Substrate A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light.)**

10. Add 100 µL of Stop Solution to every well. This stops the reaction and the plate should be read immediately.

11. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the Blank wells, manually subtract the mean optical density of the Blank wells from all readings.

CAUTION: *Bubbles in the wells will cause inaccurate readings. Ensure that all bubbles are removed prior to taking the absorbance reading.*

Calculation of Results

Manual Plotting

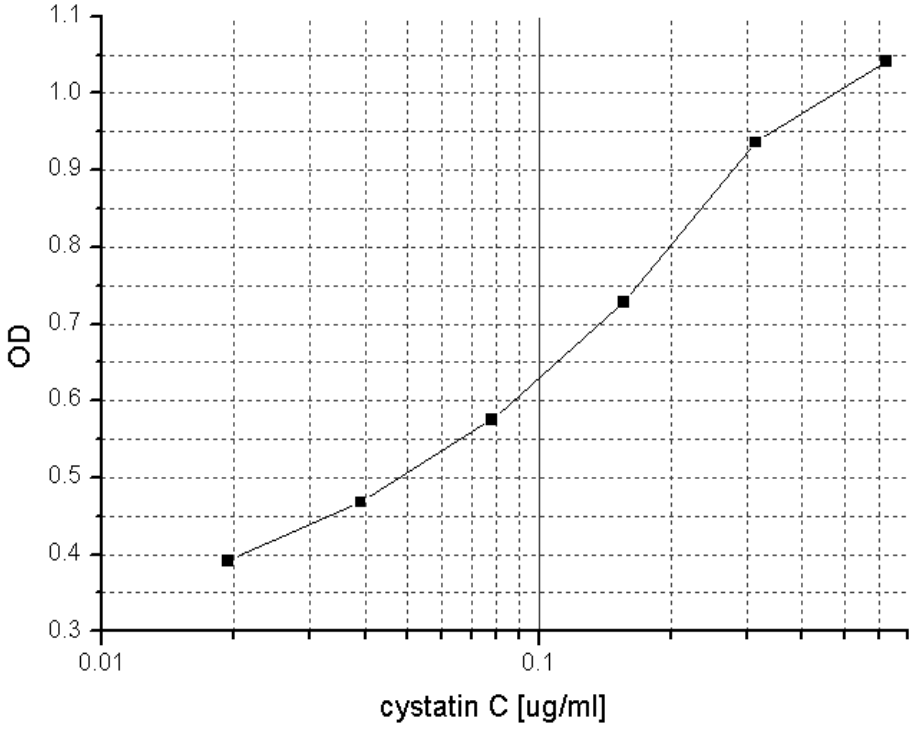
Plot the standard curve on graph paper. Known concentrations of cystatin C are plotted on the X-axis and the corresponding OD on the Y-axis. The standard curve should result in a graph that shows a direct relationship between cystatin C concentrations and the corresponding ODs. In other words, the greater the concentration of cystatin C in the sample, the higher the OD. The concentration of cystatin C in unknown samples may be determined by plotting the sample OD on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point intersects the X-axis at the concentration of cystatin C in the unknown sample.

Plate Reader/PC Interface

An alternative approach is to enter the data into a computer program curve fitting software. A good fit can be obtained with a linear regression analysis. Some data points at the top or bottom of the range tested may need to be dropped to get a good fit. Currently existing spreadsheet software can perform such plotting.

Typical Standard Curves

These curves **must not** be used to calculate cystatin C concentrations; each user must run a standard curve for each assay and version used.



Sensitivity

Sensitivity: < 19.5 ng/mL