Monkey Cystatin-C ELISA
Life Diagnostics, Inc., Catalog Number: CYSC-3

FOR RESEARCH USE ONLY

INTRODUCTION

Cystatin C is a cysteine protease inhibitor with a molecular weight of 13 kDa that is found in most body fluids. It is normally removed from blood by glomerular filtration in the kidneys, reabsorbed by the tubules and subsequently degraded. Acute kidney injury impairs this process, decreasing both glomerular filtration and tubule function. The result is an increase in both serum and urine Cystatin C levels (ref. 1). Cystatin C is thus a useful biomarker of kidney injury.

PRINCIPLE OF THE ASSAY

Test samples are diluted and incubated in microtiter wells for 45 minutes alongside prepared monkey Cystatin C standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. Cystatin C molecules are thus sandwiched between immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies. TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Optical density is measured spectrophotometrically at 450 nm. The concentration of Cystatin C is proportional to the optical density of the test sample and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti Cystatin C coated 96-well plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference standard (lyophilized)[1]
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 50 ml
- TMB Reagent (One-Step): TMB11-1, 11 ml
- Stop Solution (1N HCl): SS11-1, 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of 150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450 nm
- Graph paper (PC graphing software is optional)

STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) before use.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The Cystatin C reference standard is provided as a lyophilized stock. Reconstitute with 100 µL of distilled or deionized water ***(the reconstituted standard should be aliquoted and frozen at -20°C if future use is intended)***.
2. Label 8 polypropylene or glass tubes as 2, 1, 0.5, 0.25, 0.125, 0.063, 0.031, and 0 ng/ml.
3. Into the tube labeled 2 ng/ml, pipette the volume of diluent detailed on the reference standard vial label. Then add the indicated volume of the reference standard and mix gently. This provides the 2 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 1, 0.5, 0.25, 0.125, 0.063, 0.031, and 0 ng/ml.
5. Prepare the 1 ng/ml standard by diluting and mixing 250 µl of the 2 ng/ml standard with 250 µl of diluent in the tube labeled 1 ng/ml.
6. Similarly prepare the 0.5, 0.25, 0.125, 0.063, and 0.031 ng/ml standards by serial dilution.

SAMPLE PREPARATION

During validation studies, monkey serum was found to have a Cystatin C concentration of approximately 1 µg/ml. We therefore suggest an initial serum dilution of 1000-fold. This can be achieved with minimal use of diluent by first diluting 1 µl of serum with 99 µl of diluent to give a 100-fold dilution and then diluting 50 µl of the 100-fold diluted sample with 450 µl of diluent to give the 1000-fold diluted sample. Normal urine samples were found to have Cystatin C concentrations of approximately 50 ng/ml and were diluted 50-fold with diluent prior to assay. To eliminate matrix effects, a minimum dilution of 25-fold for urine samples is recommended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells.
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 150 rpm at room temperature (25°C) for 45 minutes.

[1] The Cystatin C standard used in this kit is of non-monkey origin. It behaves identically to monkey Cystatin C. The use of a non-monkey standard allows export of the kit without requirement for CITES documentation.

8. Wash as detailed in steps 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 50 rpm at room temperature (25°C) for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.

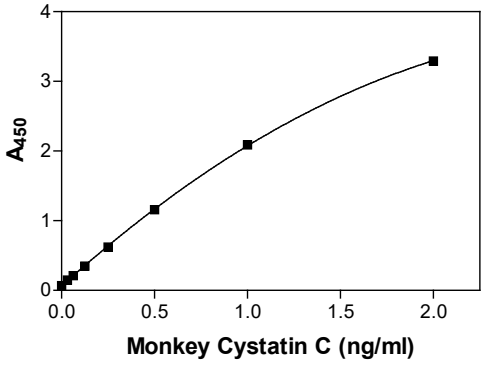
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of Cystatin C in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of Cystatin C in the sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450 nm on the Y axis against Cystatin C concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

Cystatin C (ng/ml)	Absorbance (450 nm)
2.000	3.293
1.000	2.089
0.500	1.157
0.250	0.621
0.125	0.348
0.063	0.212
0.031	0.147
0.000	0.069



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

1. B Lisowska-Myjak. Serum and urinary biomarkers of acute kidney injury. Blood Purif 29:357-365 (2010).

PROCEDURAL NOTES

1. Standards should be prepared immediately prior to use and should be used within 30 minutes of preparation.
2. Pipetting of conjugate, standards and samples into the microtiter plate should be completed within 10 minutes.
3. We recommend that standards and samples be run in duplicate.
4. It is recommended that the wells be read within 5 minutes following addition of Stop Solution.
5. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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For technical assistance please email us at
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