KAMIYA BIOMEDICAL COMPANY

Monkey Cystatin C ELISA

For the quantitative determination of cystatin C in monkey serum or urine.

Cat. No. KT-556

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Monkey Cystatin C ELISA is an enzyme immunoassay for the quantitative determination of Cystatin C in monkey serum or urine. 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. Cystatin C is thus a useful biomarker of kidney injury.

PRINCIPLE
Test samples are diluted and incubated in microtiter wells for 45 minutes alongside prepared monkey Cystatin C calibrators. 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 and 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, and 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 calibration curve.

COMPONENTS
- Anti Cystatin C coated 96-well plate (12 strips of 8 wells)
- Reference calibrator (lyophilized)
- Diluent, 50 mL
- HRP Conjugate Reagent, 11 mL
- 20X Wash Solution, 50 mL
- TMB Reagent (One-step), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Precision pipettes and tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional)
- Polypropylene or glass tubes
- Plate reader with an optical density range of 0-4 at 450 nm.
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate washer

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 de-ionized water.

CALIBRATOR PREPARATION

1. The Cystatin C reference calibrator is provided as a lyophilized stock. Reconstitute with 100 µL of distilled or de-ionized water ***(the reconstituted calibrator 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 472.3 µL of diluent. Then add 27.7 µL of the reference calibrator and mix gently. This provides the 2 ng/mL calibrator.
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 calibrator by diluting and mixing 250 µL of the 2 ng/mL calibrator 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 calibrators by serial dilution.

SAMPLE PREPARATION

During validation studies, monkey serum was found to have a Cystatin C concentration of ~1 µg/mL. We therefore suggest an initial serum dilution of 1,000-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 1,000-fold diluted sample. Normal urine samples were found to have Cystatin C concentrations of ~50 ng/mL and were diluted 50-fold with diluent prior to assay. In order to eliminate matrix effects, a minimum dilution of 25-fold for urine samples is recommended.

PROCEDURAL NOTES

1. Calibrators should be used within 30 minutes of preparation.
2. Pipetting of conjugate, calibrators, and samples into the microtiter plate should be completed within 10 minutes.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. We recommend that calibrators and samples be run in duplicate.
5. The optical density of the microtiter wells should be read within 5 minutes following the addition of Stop Solution.
6. 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.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and diluted samples into the wells.
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°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 100-150 rpm at room temperature (18-25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µL of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°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

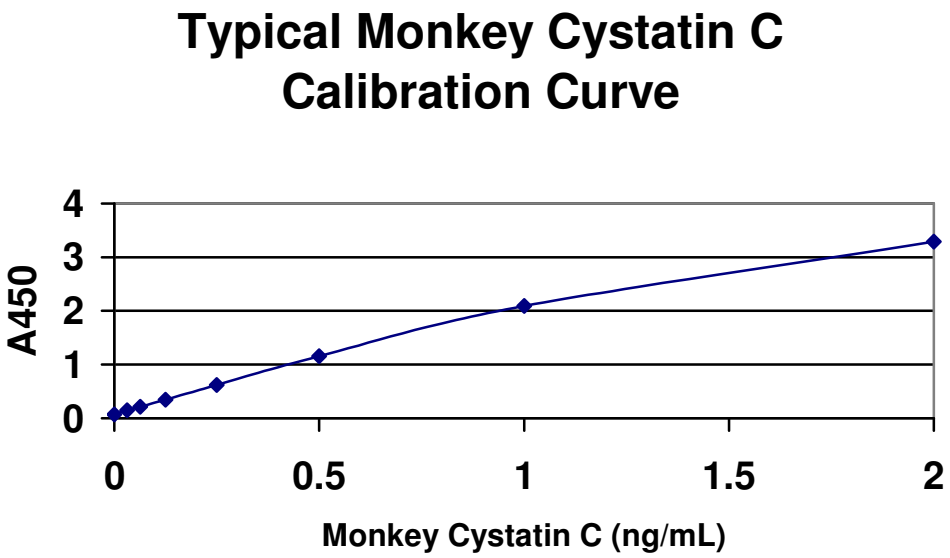
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical 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 calibration 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 the samples fall outside the calibration curve, samples should be diluted appropriately and

re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration 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 calibration curve in each experiment.

Cystatin C (ng/mL)	Absorbance (A450)
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



STORAGE

The test kit will remain stable until the expiration date provided that the components are stored at 4℃. 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 (18-25℃) before use.

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.

FOR RESEARCH USE ONLY

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