MONKEY CYSTATIN C ELISA Assay Kit

Immunoperoxidase Assay for Determination of Cystatin C in Monkey Samples

MCC91-K01

DIRECTIONS FOR USE
For Research Use Only, NOT for Diagnostic Purposes

Please Read this Package Insert Completely Before Using This Product

INTENDED USE

The Monkey Cystatin C ELISA Assay Kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring CYS in biological fluid of Monkeys.

INTRODUCTION

Cystatin C is a small cysteine proteinase inhibitor present in body fluids. Studies have shown Cystatin C levels to be directly correlated with the glomular filtration rate.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Cystatin C present in samples reacts with the anti-Cystatin C antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-CYS antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound CYS. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of CYS in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of CYS in the test sample. The quantity of CYS in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

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Anti-CYS Antibodies Bound To Solid Phase
Standards and Samples Added
CYS*Anti-CYS Complexes Formed
Unbound Sample Proteins Removed
Detection Antibody Added
Complexes Formed
Unbound Protein Removed
HRP Streptavidin Added
Complexes Formed
Unbound HRP Removed
Chromogenic Substrate Added
Determine Bound Enzyme Activity

Figure 1.

REAGENTS (Quantities sufficient for 96 determinations)

1. DILUENT (Running Buffer)
One bottle containing 60 ml of a 1X diluent running buffer.

2. WASH SOLUTION CONCENTRATE
One bottle containing 50 ml of a 20X concentrated wash solution.

3. DETECTION ANTIBODY 100X
One vial containing 150 μL of affinity purified anti-Cystatin C antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. HRP-STREPTAVIDIN 100X
One vial containing 150 μL of HRP conjugated streptavidin in a stabilizing buffer.
5. CHROMOGEN-SUBSTRATE SOLUTION
One vial containing 12 mL of 3,3’,5,5’-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

6. STOP SOLUTION
One vial containing 12 ml 0.3 M sulfuric acid.

**WARNING:** Avoid contact with skin.

7. ANTI-Cystatin C ELISA MICRO PLATE
Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-CYS antibodies.

8. Cystatin C CALIBRATOR
One vial containing a lyophilized Cystatin C calibrator. The calibrator used in this kit is of human origin. The use of a human standard allows export of this kit.

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**FOR RESEARCH USE ONLY**

REAGENT PREPARATION

1. DILUENT CONCENTRATE
Ready to use as supplied.

2. WASH SOLUTION CONCENTRATE
The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. DETECTION ANTIBODY 100X
Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 μL detection antibody to 990 μL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. HRP-STREPTAVIDIN 100X
Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 μL HPR-streptavidin to 990 μL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

5. CHROMOGEN-SUBSTRATE SOLUTION
Ready to use as supplied.

6. STOP SOLUTION
Ready to use as supplied.

7. ANTI-CYSTATIN C ELISA MICRO PLATE
Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

8. CYSTATIN C CALIBRATOR
Add 1.0 ml of distilled or de-ionized water to the CYS calibrator and mix gently until dissolved. The calibrator is now at a concentration of 1.542 ug/ml (the reconstituted calibrator should be aliquoted and frozen if future use is intended). CYS standards need to be prepared immediately prior to use (see chart below). Mix well between each step. Avoid foaming.

<table>
<thead>
<tr>
<th>Standard</th>
<th>ng/ml</th>
<th>Volume added to 1x Diluent</th>
<th>Volume of 1x Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>4</td>
<td>2 μL CYS Calibrator</td>
<td>769 μl</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>300 μl standard 6</td>
<td>300 μl</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>300 μl standard 5</td>
<td>300 μl</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>300 μl standard 4</td>
<td>300 μl</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>300 μl standard 3</td>
<td>300 μl</td>
</tr>
<tr>
<td>1</td>
<td>0.125</td>
<td>300 μl standard 2</td>
<td>300 μl</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td></td>
<td>600 μl</td>
</tr>
</tbody>
</table>

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STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. DILUENT
The 1X Diluent Concentrate is stable until the expiration date. It should be stored at 4-8°C.

2. WASH SOLUTION
The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. DETECTION ANTIBODY 100X
Undiluted Biotin conjugated anti-CYS should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.

4. HRP-STREPTAVIDIN 100X
Undiluted horseradish peroxidase conjugated streptavidin should be stored at 4-8°C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.
5. CHROMOGEN-SUBSTRATE SOLUTION
The Substrate Solution should be stored at 4-8°C and is stable until the expiration date.

6. STOP SOLUTION
The Stop Solution should be stored at 4-8°C and is stable until the expiration date.

7. ANTI- CYSTATIN C ELISA MICRO PLATE
Anti-CYS coated wells are stable until the expiration date, and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

8. CYSTATIN C CALIBRATOR
The lyophilized Cystatin C calibrator should be stored at 4C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (Avoid multiple freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use.

INDICATIONS OF INSTABILITY
If the test is performing correctly, the results observed with the standard solutions should be within 20 % of the expected values.

SPECIMEN COLLECTION AND HANDLING
Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

1. Precautions
For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives
No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

3. Known interfering substances
Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

MATERIAL PROVIDED
See "REAGENTS"

MATERIALS REQUIRED
BUT NOT PROVIDED
- Precision pipette (2 μL to 200 μL) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H₂O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Orbital Shaker

ASSAY PROTOCOL

DILUTION OF SAMPLES
The assay for quantification of CYS in samples requires that each test sample be diluted before use. For a single step determination a dilution at 1/1,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

1. To prepare a 1/1,000 dilution of sample, transfer 2 μL of sample to 198 μL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 30 μL of sample to 270 μL of 1X diluent. This gives you a 1/1,000 dilution. Mix thoroughly at each stage. Dilute samples immediately prior to use.

PROCEDURE

1. Bring all reagents to room temperature before use.

2. Pipette 100 μL of
   Standard 0 (0.0 ng/ml) in duplicate
   Standard 1 (0.125 ng/ml) in duplicate
   Standard 2 (0.25 ng/ml) in duplicate
   Standard 3 (0.5 ng/ml) in duplicate
   Standard 4 (1 ng/ml) in duplicate
   Standard 5 (2 ng/ml) in duplicate
   Standard 6 (4 ng/ml) in duplicate

3. Pipette 100 μL of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate while shaking on an orbital shaker at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 μL of appropriately diluted detection antibody to each well. Incubate the micro titer plate while shaking on an orbital shaker at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5/6.

9. Pipette 100 μL of appropriately diluted HRP-streptavidin to each well. Incubate the micro titer plate while shaking on an orbital shaker at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

10. Wash and blot the wells as described in Steps 5/6.

11. Pipette 100 μL of TMB Substrate Solution into each well.

12. Incubate in the dark while shaking on an orbital shaker at room temperature for precisely ten (10) minutes.

13. After ten minutes, add 100 μL of Stop Solution to each well.

14. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer’s specifications.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the Cystatin C concentration in original samples.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.

3. Do not mix or substitute reagents with those from other lots or sources.
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