Performance characteristics, continued

Expected values

<table>
<thead>
<tr>
<th>Range (ng/mL)</th>
<th>Average (ng/mL)</th>
<th>Reference Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>522–906</td>
<td>675</td>
<td>590–910</td>
</tr>
</tbody>
</table>

Linearity of dilution

Linearity was determined by assaying high and low concentration samples mixed in the ratios shown in the following table.

<table>
<thead>
<tr>
<th>High Sample %</th>
<th>Low Sample %</th>
<th>Expected Conc. (ng/mL)</th>
<th>Observed Conc. (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>20</td>
<td>Serum: 10.84 3.30 11.64 3.57 105.6 108.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>Serum: 8.36 2.89 9.15 2.90 97.7 100.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td>Serum: 7.87 2.67 7.77 2.42 98.5 97.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>Serum: 6.41 2.06 5.80 1.82 90.4 86.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity

The analytical sensitivity of the assay is 0.058 ng/ml human cystatin C. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Specificity

This assay has been shown to detect cystatin C from human samples only. Do not use the kit for non-human samples.

Serum samples from dog, monkey, rat and mouse were serially diluted in 1X Assay Buffer and no reactivity was detected.

Materials required but not supplied

- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Distilled or deionized water
- Kits are supplied in amber tubes. Redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the horseradish peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.

Procedural guidelines

- Distilled or deionized water
- Microtiter plate washer with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm).
- Plate washer–automated or manual (syringe bottle, manifold dispenser, or equivalent).
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

For research use only. Not for use in diagnostic procedures.
Prepare 1X Wash Buffer
1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer
1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Sample preparation guidelines
• Refer to the ELISA Technical Guide at thermofisher.com for detailed sample preparation procedures.
• Collect samples in pyrogen/endotoxin-free tubes.
• Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
• Avoid the use of hemolyzed or lipemic sera.
• Use all samples within 2 hours of dilution.

Pre-dilute samples
Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Use all samples within 2 hours of dilution.
• Dilute serum and plasma samples ≥1:50 in 1X Assay Buffer.
• Dilute urine samples ≥1:4 with 1X Assay Buffer.
• Dilute tissue culture media samples with the corresponding cell culture medium.

Dilute standards
Note: Use glass or plastic tubes for diluting standards.
Important! For tissue culture media samples, dilute standards with the appropriate tissue culture medium instead of 1X Assay Buffer.
1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 15 µL Cystatin C Standard to one tube containing 585 µL 1X Assay Buffer and label as 10 ng/mL Hu cystatin C.
3. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 ng/mL Hu cystatin C.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Use the standards within 2 hours of preparation.

Perform ELISA (Total assay time: 2.0 hours)
IMPORTANT! Perform a standard curve with each assay.

Dilution diagram

<table>
<thead>
<tr>
<th>Standard Hu Cystatin C (ng/mL)</th>
<th>Optical Density (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.595</td>
</tr>
<tr>
<td>5</td>
<td>0.810</td>
</tr>
<tr>
<td>2.5</td>
<td>0.399</td>
</tr>
<tr>
<td>1.25</td>
<td>0.221</td>
</tr>
<tr>
<td>0.625</td>
<td>0.129</td>
</tr>
<tr>
<td>0.313</td>
<td>0.082</td>
</tr>
<tr>
<td>0.156</td>
<td>0.047</td>
</tr>
<tr>
<td>0</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Performance characteristics
Standard curve (example) The following data were obtained for the various standards over the range of 0–10 ng/mL human cystatin C.

Inter-assay precision
Samples were independently run three times in ten assays in triplicate to determine precision between assays.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>7.79</td>
<td>5.08</td>
<td>4.97</td>
<td>1.04</td>
</tr>
<tr>
<td>%CV</td>
<td>8.4</td>
<td>10.2</td>
<td>11.1</td>
<td>12.4</td>
</tr>
</tbody>
</table>

CV = Coefficient of Variation

Intra-assay precision
Samples of known human cystatin C concentration were assayed in replicates of 20 to determine precision within an assay.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>7.72</td>
<td>5.08</td>
<td>4.42</td>
<td>0.88</td>
</tr>
<tr>
<td>%CV</td>
<td>9.1</td>
<td>10.3</td>
<td>7.6</td>
<td>10.3</td>
</tr>
</tbody>
</table>

CV = Coefficient of Variation

Add Pre-dilute Standard to 1X Assay Buffer

Add Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Add detection antibody
a. Add 50 µL of detection antibody to each well. Incubate for 30 minutes at room temperature.
               b. Incubate for 30 minutes at room temperature.
               c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 300 µL of 1X Wash Buffer.

Add chromogen
a. Add 50 µL Cystatin C Conjugate into each well.
               b. Incubate for 30 minutes at room temperature.
               c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 300 µL of 1X Wash Buffer.

Add chromogen
a. Add 50 µL chromogen to each well. The substrate solution will begin to turn blue.
               b. Incubate for 30 minutes at room temperature.
               c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 300 µL of 1X Wash Buffer.

Add stop solution
Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Add chromogen
a. Add 50 µL chromogen to each well. The substrate solution will begin to turn blue.
               b. Incubate for 30 minutes at room temperature.
               c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 300 µL of 1X Wash Buffer.

Add stop solution
Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Add Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Add Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.

Add Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells changes from blue to yellow.
Prepare 1X Wash Buffer
1. Dilute 14 mL of Assay Buffer (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Prepare 1X Assay Buffer
1. Dilute 15 mL of Wash Solution Concentrate (20X) with 285 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. Use the diluted buffer within 3 months.

Dilute standards
Note: Dilute standards with the appropriate tissue culture medium instead of 1X Assay Buffer.
1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 15 µL Cystatin C Standard to one tube containing 585 µL 1X Assay Buffer and label as 10 ng/mL Hu cystatin C.
3. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 ng/mL Hu cystatin C.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. Use the standards within 2 hours of preparation.

Sample preparation guidelines
- Refer to the ELISA Technical Guide at thermofisher.com for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Discard samples with the corresponding cell culture medium.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- Pre-dilute samples
- Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

Perform ELISA
Total assay time: 2.0 hours
1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

Performance characteristics
Standard curve
The following data were obtained for the various standards over the range of 0–10 ng/mL human cystatin C.

<table>
<thead>
<tr>
<th>Standard Hu Cystatin C (ng/mL)</th>
<th>Optical Density (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.595</td>
</tr>
<tr>
<td>5</td>
<td>0.810</td>
</tr>
<tr>
<td>2.5</td>
<td>0.399</td>
</tr>
<tr>
<td>1.25</td>
<td>0.221</td>
</tr>
<tr>
<td>0.625</td>
<td>0.129</td>
</tr>
<tr>
<td>0.313</td>
<td>0.082</td>
</tr>
<tr>
<td>0.156</td>
<td>0.017</td>
</tr>
<tr>
<td>0</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Intra-assay precision
Samples of known human cystatin C concentration were assayed in triplicate to determine precision between assays.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>7.72</td>
<td>5.08</td>
<td>4.97</td>
<td>1.04</td>
</tr>
<tr>
<td>%CV</td>
<td>8.4</td>
<td>10.2</td>
<td>11.1</td>
<td>12.4</td>
</tr>
</tbody>
</table>

CV = Coefficient of Variation

Inter-assay precision
Samples were independently run three times in ten assays in duplicate to determine precision between assays.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (ng/mL)</td>
<td>7.72</td>
<td>5.08</td>
<td>4.97</td>
<td>1.04</td>
</tr>
<tr>
<td>%CV</td>
<td>9.1</td>
<td>10.3</td>
<td>7.6</td>
<td>13.8</td>
</tr>
</tbody>
</table>

CV = Coefficient of Variation
Performance characteristics, continued

Expected values

Thirteen random human serum samples were tested in the assay.

<table>
<thead>
<tr>
<th>Range (ng/mL)</th>
<th>Average (ng/mL)</th>
<th>Reference Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.22–9.96</td>
<td>4.25</td>
<td>0.25–9.90</td>
</tr>
</tbody>
</table>

Linearity of dilution

Linearity was determined by assaying high and low concentration samples mixed in the ratios shown in the following table.

<table>
<thead>
<tr>
<th>High Sample %</th>
<th>Low Sample %</th>
<th>Expected Conc. (ng/mL)</th>
<th>Observed Conc. (ng/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>20</td>
<td>Serum: 10.88, 3.30</td>
<td>Serum: 11.46, 3.57</td>
<td>105.6, 108.1</td>
</tr>
<tr>
<td>60</td>
<td>40</td>
<td>Serum: 9.13, 2.89</td>
<td>Serum: 9.15, 2.90</td>
<td>97.7, 100.4</td>
</tr>
<tr>
<td>80</td>
<td>40</td>
<td>Serum: 7.87, 2.67</td>
<td>Serum: 7.77, 2.42</td>
<td>98.5, 97.9</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>Serum: 5.91, 2.06</td>
<td>Serum: 5.91, 1.82</td>
<td>99.4, 98.5</td>
</tr>
</tbody>
</table>

Mean Recovery: 98.1% ± 1.5%

Specificity

This assay has been shown to detect cystatin C from human samples only. Do not use the kit for non-human samples. Serum samples from dog, monkey, rat and mouse were serially diluted in 1X Assay Buffer and no reactivity was detected.

Sensitivity

The analytical sensitivity of the assay is 0.058 ng/mL human cystatin C. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies’ General Terms and Conditions of Sale found on Life Technologies’ website at thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

Manufacturers address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For support visit thermofisher.com/support or email techsupport@lifetech.com.

thermofisher.com

2 January 2017

For research use only. Not for use in diagnostic procedures.

Human Cystatin C Immunoassay Kit
Catalog Number EIA06179 (96 tests)
Rev 1.0

For safety and biohazard guidelines, see the “Safety” appendix in the ELISA Technical Guide (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Human Cystatin C Immunoassay Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human cystatin C in serum, EDTA and heparin plasma, urine and tissue culture media. The assay recognizes both natural and recombinant human cystatin C.

Cystatin C is a non-glycosylated 13 kDa protein belonging to the cystatin superfamily. Cystatin C is a secreted protein produced at a constant rate in all nucleated cells, and thus found in detectable amounts in most body fluids. Cystatin C is removed from blood plasma by glomerular filtration in the kidneys. There is a linear relationship between the reciprocal cystatin C concentration in plasma and the constant rate in all nucleated cells, and thus found in detectable amounts in most body fluids. Cystatin C serum concentration is not affected by factors such as age, gender and body mass.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components

- Cystatin C Standard; 400 ng/mL native Hu Cystatin C
- Assay Buffer Concentrate (5X)
- Hu Cystatin C Antibody Coated Wells, 96-well strip-well plate
- Cystatin C Conjugate
- Wash Buffer Concentrate [20X]
- TMB (Tetramethylbenzidine) Substrate
- Stop Solution; contains 1 M HCl
- Plate Sealer

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm (preferably with correction between 570 nm and 590 nm).
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Plate washing

Procedural guidelines

- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- Solutions containing sodium azide will inhibit the activity of the peroxidase conjugate. Ensure that there is no contamination of labware or the plate washer with azide containing solutions.