ab211099
Cysteine Assay Kit
(Fluorometric)

For the sensitive and accurate measurement of Cysteine in serum and urine.

This product is for research use only and is not intended for diagnostic use.
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1. Overview

Cysteine Assay Kit (Fluorometric) (ab211099) provides a convenient method to quantify cysteine present in biological fluids such as serum and plasma. The assay principle is based on the cleavage of thiol group in reduced cysteine to produce that emits a stable signal that can be detected at Ex/Em = 365/450 nm. The amount of signal is directly proportional to the amount of total cysteine in the sample. The reaction is specific and other thiol-based amino acids do not interfere with the assay. The assay can detect as little as 10 µM of Cysteine in a variety of samples.

Cysteine (CYS, C) is a sulfhydryl-containing amino acid, and is an important structural and functional part of proteins. In animals, cysteine is synthesized from trans-sulfuration of homocysteine (HCY), which is itself derived from metabolism of the amino acid methionine. The enzyme Cystathionine β-Synthase catalyzes condensation of homocysteine with serine to form cystathionine, which is deaminated and hydrolyzed by Cystathionine β-lyase to form cysteine and α-ketobutyrate. Due to its nucleophilic nature, the thiol group of cysteine has numerous biological functions. The formation of disulfide linkages between the thiol groups of cysteine residues helps to stabilize the tertiary and quaternary structure of proteins. Cysteine, homocysteine and other aminothiols exist in plasma in reduced, oxidized, and protein-bound forms, interacting with each other through redox pathways. Cysteine is the limiting precursor of the major intracellular antioxidant glutathione. The individuals with lower cysteine levels are more prone to damage from reactive oxygen species, which are generally removed either by thiols or by glutathione-linked enzymes. An elevated level of total cysteine also predicts adverse outcomes such as cardiovascular diseases and metabolic syndromes.
2. Protocol Summary

Standard curve preparation

↓

Sample preparation

↓

Add reaction mix and incubate for 30 minutes at 37°C

↓

Add Enzyme Mix II and incubate for 5 minutes at 37°C

↓

Add CYS Probe and mix

↓

Measure fluorescence at Ex/Em = 365/450 nm immediately in kinetic mode for 5-30 minutes at 37°C

*For kinetic mode detection, incubation time given in this summary is for guidance only
3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

⚠️ Note: Reconstituted components are stable for two months.
5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage temperature (before prep)</th>
<th>Storage temperature (after prep)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYS Assay Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>4°C / -20°C</td>
</tr>
<tr>
<td>Enzyme Mix I</td>
<td>50 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Enzyme Mix II (500 mg)</td>
<td>3 x 1 vial</td>
<td>-20°C</td>
<td>4°C</td>
</tr>
<tr>
<td>Reducing Agent (20 mg)</td>
<td>2 x 1 vial</td>
<td>-20°C</td>
<td>4°C</td>
</tr>
<tr>
<td>HCY Blocker</td>
<td>100 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>CYS Probe</td>
<td>500 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>CYS Standard (10 µmole)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
7. **Materials Required, Not Supplied**

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 365/450 nm
- MilliQ water or other type of double distilled water (ddH₂O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well white plate with clear flat bottom
8. Technical Hints

- This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.
9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 CYS Assay Buffer (25 mL):
Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 Enzyme Mix I (50 µL):
Ready to use as supplied. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C. Freeze/thaw should be limited to two times. Keep on ice during use.

9.3 Enzyme Mix II (lyophilized, 500 mg):
Reconstitute each vial in 1 mL of CYS Assay Buffer as needed. Store at 4°C. Use the reconstituted Enzyme Mix II within a week. Keep on ice during use.

9.4 Reducing Agent (lyophilized, 20 mg):
Reconstitute each vial in 220 µL of CYS Assay Buffer as needed. Store at 4°C. Use the reconstituted Enzyme Mix II within a week. Keep on ice during use.

9.5 HCY Blocker (100 µL):
Ready to use as supplied. Equilibrate to room temperature. Aliquot blocker so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw.

9.6 CYS Probe (500 µL):
Ready to use as supplied. Equilibrate to room temperature before use. Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light.

9.7 CYS Standard (lyophilized, 10 µmole):
Reconstitute in 900 µL ddH$_2$O to generate 10 mM CYS Standard solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw. Use within two months.
10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 1 mM CYS Standard by diluting 10 µL of 10 mM CYS Standard to 90 µL of ddH$_2$O.

10.2 Using 10 mM CYS Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

<table>
<thead>
<tr>
<th>Standard #</th>
<th>CYS Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End amount CYS in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>30</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>24</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>18</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>12</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (2 x 10 µL).
11. Sample Preparation

General sample information:
- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Biological fluids (serum, urine and other):
11.1.1 Centrifuge sample at 10,000 xg in a cold centrifuge at 4°C for 5 minutes.
11.1.2 Collect the supernatant.

△ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.
12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Set up reaction wells:
- Standard wells = 10 µL standard dilutions.
- Sample wells = 5 – 10 µL sample (adjust volume to 10 µL/well with CYS Assay Buffer).
- Reagent Background Control wells = 10 µL CYS Assay Buffer.

12.2 CYS Reaction mix:

12.2.1 Prepare 1:10 dilution of Enzyme Mix I by adding 2 µL Enzyme Mix I to 18 µL CYS Assay Buffer. Make as much as needed. Mix enough reagents for the total number of wells to be assayed.

12.2.2 Prepare 200 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYS Assay Buffer</td>
<td>193</td>
</tr>
<tr>
<td>Diluted (1:10) Enzyme Mix I</td>
<td>5</td>
</tr>
<tr>
<td>Reducing Agent</td>
<td>1</td>
</tr>
<tr>
<td>HCY Blocker</td>
<td>1</td>
</tr>
</tbody>
</table>

12.2.3 Add 200 µL of Reaction Mix to each well. Mix well using a multichannel pipette.

12.2.4 Incubate at 37°C for 30 minutes.

12.2.5 Add 30 µL of Enzyme Mix II to each well. Mix well using a multichannel pipette.

12.2.6 Incubate at 37°C for 5 minutes.

Note: Incubation time for both the Standard and the sample wells must be consistent.
12.3 Plate measurement:
12.3.1 After incubation, add 5 µL of CYS Probe to each well. Mix well.
12.3.2 Measure immediately fluorescence at Ex/Em = 365/450 nm in kinetic mode for at least 30 minutes at room temperature.

△ Note: We recommend measuring fluorescence in a kinetic mode, and choosing two time points (T1 and T2) to calculate the CYS amount in the sample. The Cysteine Standard curve should be read along with the samples.
13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:
13.1.1 Subtract the mean fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected fluorescence.
13.1.2 Average the duplicate reading from each standard.
13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of Cysteine (CYS) in the sample:
13.2.1 Subtract the reagent background control from sample reading.
13.2.2 For all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding fluorescence values at those points (RFU1 and RFU2).
13.2.3 Calculate $\Delta$RFU for sample as follows:

$\Delta$RFU = RFU2 – RFU1

13.2.4 Apply $\Delta$RFU to the CYS Standard curve to get Cysteine (B) amount generated during the reaction.
13.2.5 Concentration of Cysteine (nmol/µL or mM) in the test samples is calculated as:

Cysteine concentration = $\frac{B}{V} \times D$

Where:
B = amount of Cysteine (CYS) in the sample well calculated from standard curve (nmol).
V = sample volume added in the sample wells (µL).
D = sample dilution factor
14. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Figure 1. Typical Cysteine standard calibration curve.
Figure 2. Kinetic curves showing estimation of Cysteine concentration present in human plasma. Calculated cysteine concentration in plasma following assay protocol is 251 ± 20 µM.
15. Quick Assay Procedure

△ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents; get equipment ready.
- Prepare CYS standard dilution [2 – 10 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (10 µL), samples (10 µL) and reagent background control wells (10 µL).
- Prepare a master mix for Cysteine Reaction Mix:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYS Assay Buffer</td>
<td>193</td>
</tr>
<tr>
<td>Diluted (1:10) Enzyme Mix I</td>
<td>5</td>
</tr>
<tr>
<td>Reducing Agent</td>
<td>1</td>
</tr>
<tr>
<td>HCY Blocker</td>
<td>1</td>
</tr>
</tbody>
</table>

- Add 200 µL of Reaction mix to Standard, sample and reagent background control wells.
- Incubate at 37°C for 30 minutes.
- Add 30 µL of Enzyme Mix II.
- Incubate at 37°C for 5 minutes.
- Add 5 µL of CYS Probe.
- Measure fluorescence immediately at Ex/Em = 365/450 nm in kinetic mode for at least 30 minutes at room temperature.
## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay not working</td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at assay temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
</tbody>
</table>
|          | Use of a different microplate        | Colorimetric: clear plates  
Fluorometric: black wells/clear bottom plates  
Luminometric: white wells/clear bottom plates |
<p>| Sample with erratic readings | Cells/tissue samples not homogenized completely | Use Dounce homogenizer, increase number of strokes                        |
|          | Samples used after multiple free/ thaw cycles | Aliquot and freeze samples if needed to use multiple times               |
|          | Use of old or inappropriately stored samples | Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use |
|          | Presence of interfering substance in the sample | Check protocol for interfering substances; deproteinize samples           |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use                   |
|          | Allowing reagents to sit for extended times on ice | Always thaw and prepare fresh reaction mix before use                      |
|          | Incorrect incubation times or temperatures | Verify correct incubation times and temperatures in protocol               |</p>
<table>
<thead>
<tr>
<th>Problem</th>
<th>Reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard readings do not follow a linear pattern</strong></td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix whenever possible</td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions described in the protocol</td>
</tr>
<tr>
<td><strong>Unanticipated results</strong></td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear range</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. Notes
Technical Support

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