Version 5b, Last updated 27 September 2023

ab211099
Cysteine Assay Kit (Fluorometric)

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

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Cysteine Assay Kit (Fluorometric) (ab211099) provides a convenient method to quantify cysteine present in biological fluids such as serum, plasma and urine. 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 a-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 CYS Converter Mix/Enzyme Mix II and incubate for 5 minutes at 37°C

Add H2S Probe/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.

Δ 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

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Cysteine Assay Buffer/CYS Assay Buffer	25 mL	-20°C	4°C / -20°C
CYS Enzyme Mix/Enzyme Mix I	50 µL	-20°C	-20°C
CYS Converter Mix/Enzyme Mix II (500 mg)	3 x 1 vial	-20°C	4°C
Reducing Agent I/Reducing Agent (20 mg)	2 x 1 vial	-20°C	4°C
HCY Blocker	100 µL	-20°C	-20°C
H2S Probe/CYS Probe	500 µL	-20°C	-20°C
Cysteine Standard/CYS Standard (10 µmole)	1 vial	-20°C	-20°C

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 (ddH_2O)
- 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/black 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 Cysteine Assay Buffer/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 CYS Enzyme Mix/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 CYS Converter Mix/Enzyme Mix II (lyophilized, 500 mg):

Reconstitute each vial in 1 mL of Cysteine Assay Buffer/CYS Assay Buffer as needed. Store at 4°C. Use the reconstituted CYS Converter Mix/Enzyme Mix II within a week. Keep on ice during use.

9.4 Reducing Agent I/Reducing Agent (lyophilized, 20 mg):

Reconstitute each vial in 220 µL of Cysteine Assay Buffer/CYS Assay Buffer as needed Store at 4°C. Use the reconstituted Reducing Agent I/Reducing Agent 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 H2S Probe/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 Cysteine Standard/CYS Standard (lyophilized, 10 µmole):

Reconstitute in 900 µL ddH_2O to generate 10 mM Cysteine Standard/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 Cysteine Standard/CYS Standard by diluting 10 µL of 10 mM Cysteine Standard/CYS Standard to 90 µL of ddH_2O.

10.2 Using 1 mM Cysteine Standard/CYS Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Cysteine Standard/CYS Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount CYS in well (nmol/well)
1	0	30	10	0
2	6	24	10	2
3	12	18	10	4
4	18	12	10	6
5	24	6	10	8
6	30	0	10	10

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.
- Use Cysteine Assay Buffer/CYS Assay Buffer to dilute sample to the required volume.

11.1 Biological fluids (serum, plasma, urine and other):

11.1.1 Centrifuge sample at 10,000 x*g* in a cold centrifuge at 4°C for 5 minutes.

11.1.2 Collect the supernatant.

Δ 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 Cysteine Assay Buffer/CYS Assay Buffer).
- Reagent Background Control wells =10 µL Cysteine Assay Buffer/CYS Assay Buffer.

12.2 CYS Reaction mix:

12.2.1 Prepare 1:10 dilution of CYS Enzyme Mix/Enzyme Mix I by adding 2 µL CYS Enzyme Mix/Enzyme Mix I to 18 µL Cysteine Assay Buffer/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:

Component	Reaction Mix (µL)
Cysteine Assay Buffer/CYS Assay Buffer	193
Diluted (1:10) CYS Enzyme Mix/Enzyme Mix I	5
Reducing Agent I/Reducing Agent	1
HCY Blocker	1

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 CYS Converter Mix/Enzyme Mix II to each well. Mix well using a multichannel pipette.

12.2.6 Incubate at 37°C for 5 minutes.

Δ **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 H2S Probe/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.

Δ 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 Average the duplicate readings (RFU) from each standard and sample.

13.2 Subtract the RFU of reagent background control from all sample RFU to get the corrected RFU of samples.

13.3 For all reactions wells, plot the RFU versus time (min).

13.4 For all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding RFU at those points (RFU1 and RFU2).

13.5 Calculate the ΔRFU for standards and samples as follows:
ΔRFU = RFU2 – RFU1.

13.6 For all standards, plot the ΔRFU versus the amount of Cysteine (CYS) in each well (nmol/well). Draw the line of the best fit to construct the standard curve (Excel can do this step). Calculate the trendline equation based on your standard curve (use the equation that provides the most accurate fit).

13.7 For all samples, apply ΔRFU to the standard curve trendline equation to get Cysteine (B) amount present in each well.

13.8 Concentration of Cysteine (nmol/ µL or mM) in the test samples is calculated as:

$$\text{Cysteine concentration} = \frac{B}{V} * D$$

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.

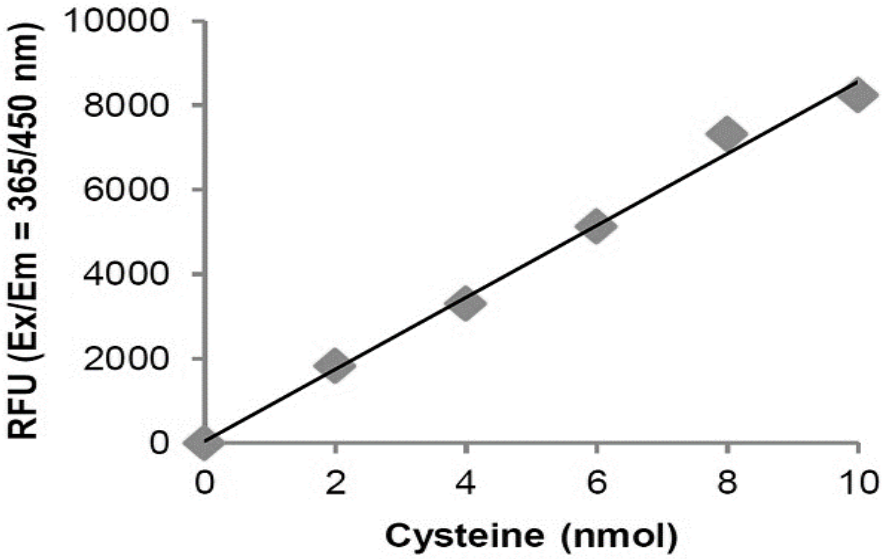

Figure 1. Typical Cysteine standard calibration curve.

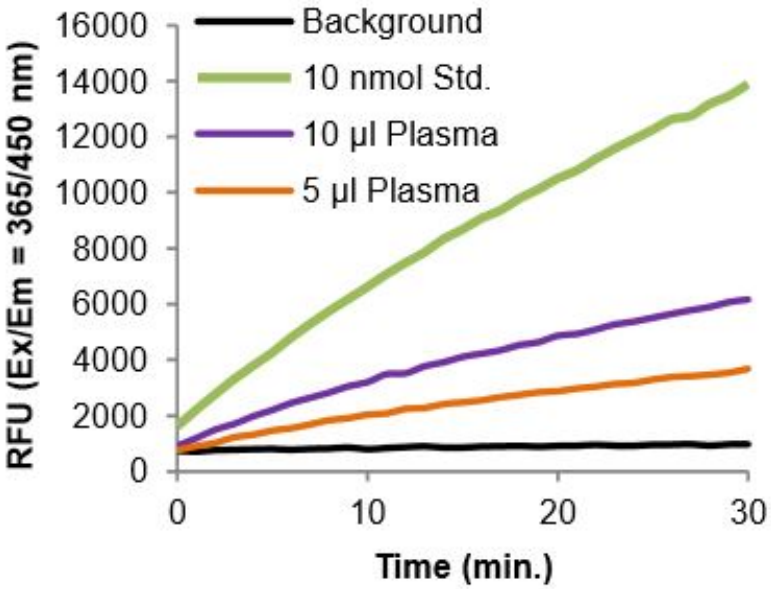

Figure 2. Kinetic curves showing estimation of Cysteine concentration present in human plasma. Calculated cysteine concentration in plasma following assay protocol is 251 ± 20 µM.

15. Quick Assay Procedure

Δ 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 Cysteine Standard/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:

Component	Reaction Mix (µL)
Cysteine Assay Buffer/CYS Assay Buffer	193
Diluted (1:10) CYS Enzyme Mix/Enzyme Mix I	5
Reducing Agent I/Reducing Agent	1
HCY Blocker	1

- 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 CYS Converter Mix/Enzyme Mix II.
- Incubate at 37°C for 5 minutes.
- Add 5 µL of H2S Probe/CYS Probe.
- Measure fluorescence immediately at Ex/Em = 365/450 nm in kinetic mode for at least 30 minutes at room temperature.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: white/black wells/clear bottom plates Luminometric: white wells/clear bottom plates
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

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)