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Cysteine Assay Kit (Fluorometric)

rev 11/19

(Catalog # K558-100; 100 assays; Store at -20°C)

I. Introduction:

Cysteine (CYS) is a sulfhydryl-containing amino acid and an important structural and functional part of proteins. In animals, cysteine is synthesized from transsulfuration of homocysteine, which is itself derived from metabolism of the amino acid methionine. 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. Because of 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 (HCY), 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. BioVision's Cysteine Assay Kit provides a simple, sensitive, and high-throughput adaptable assay that detects physiological concentration of cysteine in a variety of biological fluids. The principle of the assay is based on the cleavage of thiol group of reduced cysteine producing a fluorophore (Ex/Em = 365/450 nm) with a stable signal, which is directly proportional to the amount of total cysteine in the sample. The assay 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.

II. Application:

- Estimation of Cysteine in various biological samples

III. Sample Type:

- Serum, plasma etc.

IV. Kit Contents:

Components	K558-100	Cap Code	Part Number
CYS Assay Buffer	25 ml	WM	K558-100-1
Enzyme Mix I	50 µl	Green	K558-100-2
Enzyme Mix II	3 vials	Blue	K558-100-3
Reducing Agent	2 vials	Yellow	K558-100-4
HCY Blocker	100 µl	White	K558-100-5
CYS Probe	0.5 ml	Purple	K558-100-6
CYS Standard	1 vial	Red	K558-100-7

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. We recommend white plate for this assay.
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly spin small vials prior to opening. Read the entire protocol before performing the assay.

- **CYS Assay Buffer:** Store at -20°C or 4°C. Bring to room temperature (RT) before use.
- **Enzyme Mix I:** Aliquot and store at -20°C. Freeze/thaw should be limited to two times. Keep on ice during use.
- **Enzyme Mix II:** Reconstitute each vial with 1 ml of CYS Assay Buffer as needed. Store at 4°C. Keep on ice during use. Use the reconstituted Enzyme Mix II within a week.
- **Reducing Agent:** Reconstitute each vial with 220 µl of CYS Assay Buffer as needed. Store at 4°C. Keep on ice during use. Use the reconstituted Reducing Agent within a week.
- **HCY Blocker:** Bring to RT. Aliquot and store at -20°C. Avoid repeated freeze/thaw.
- **CYS Probe:** Light sensitive. Store at -20°C. Bring to RT before use.
- **CYS Standard:** Reconstitute with 1.26 ml dH_2O to generate 10 mM L-Cysteine Standard solution. Aliquot and Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

VII. Cysteine Assay Protocol:

1. **Sample Preparation:** Centrifuge biological fluids at 10,000 X g, 4°C for 5 min. Collect the supernatant & add 5-10 µl into desired well(s) in a 96-well plate. Adjust the volume to 10 µl/well with CYS Assay Buffer. Add 10 µl CYS Assay Buffer to one well as reagent background control.
2. **Standard Curve Preparation:** Prepare 1 mM Cysteine Standard by adding 10 µl of 10 mM CYS Standard to 90 µl of ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of 1 mM Cysteine Standard into a series of wells in a 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmol of cysteine/well. Adjust the volume to 10 µl/well with CYS Assay Buffer.
3. **Reaction Mix:** Dilute Enzyme Mix I 10-fold 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. For each well, prepare 200 µl of Reaction Mix containing:

	Reaction Mix
CYS Assay Buffer	193 µl
Diluted Enzyme Mix I	5 µl
Reducing Agent	1 µl
HCY Blocker	1 µl

Mix well. Add 200 µl of Reaction Mix to Standard, sample and reagent background control wells. Mix well using a multichannel pipette and incubate at 37°C for 30 min. Add 30 µl of Enzyme Mix II. Mix well using a multichannel pipette and incubate for 5 min at 37°C.

Notes:

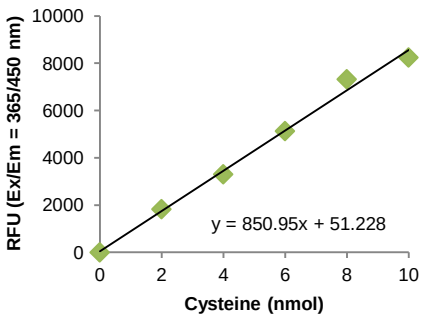
(a) Follow protocol exactly as described. Any deviations can result in sub-optimal results.
(b) Incubation time for both the Standard and the sample wells must be consistent.

4. **Measurement:** After incubation, add 5 µl of CYS Probe. Mix and measure fluorescence (Ex/Em = 365/450 nm) in kinetic mode for at least 30 min. at RT. Choose two time points (T1 & T2) in the linear range (can be as short as 2 min.) of plot and obtain corresponding RFU for sample (RS1 and RS2). Read the Cysteine Standard Curve along with the samples.
5. **Calculation:** Subtract 0 Standard reading from all Standard readings. Plot the CYS Standard Curve. Subtract reagent background control reading from sample reading. Apply the ΔRFU to the Standard Curve to get B nmoles of cysteine generated during the reaction.

Sample CYS Concentration (C) = B/V nmol/µl or mM

Where: **B** is amount of cysteine in the sample well from Standard Curve (nmol)
V is sample volume added into the reaction well (µl)

(a)



(b)

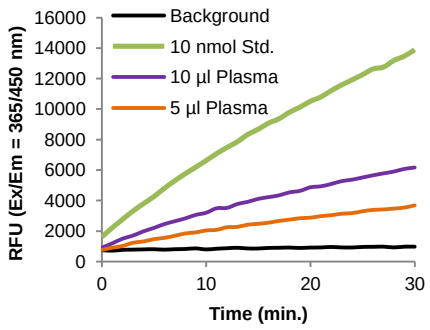


Figure: (a) Cysteine Standard Curve. **(b)** Estimation of Cysteine concentration in human plasma following the kit protocol. Calculated cysteine concentration in plasma is 251 ± 20 µM.

VIII. Related Products:

Glycine Assay Kit (K589-100)
Alanine Colorimetric/Fluorometric Assay Kit (K652)
Phenylalanine Fluorometric Assay Kit (K572)
Cystathionine β Synthase, human recombinant (7844)
Active AHCY, human recombinant (7527)
Glutamate Colorimetric Assay Kit (K629)
Aspartate Colorimetric/Fluorometric Assay Kit (K552)
Tyrosine Colorimetric Assay Kit (K573)
L-Methionine γ-lyase, bacterial recombinant (7848)
Glycine Oxidase, bacterial recombinant (7845)

FOR RESEARCH USE ONLY! Not to be used on humans.