CREATIVE BIOMART®
Assay Kit

Cysteine Assay Kit (Fluorometric)

Product Information

Cat.No. Kit-1003

Product Overview

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.

Storage

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

Shipping

Gel Pack

Size

100 assays

Kit Components

CYS Assay Buffer 25 ml;
Enzyme Mix I 50 µl;
Enzyme Mix II 3 vials;
Reducing Agent 2 vials;
HCY Blocker 100 µl;
CYS Probe 0.5 ml;
CYS Standard 1 vial
Probe; CYS Standard

Materials Required but Not Supplied

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

Compatible Sample Types

Serum, plasma etc.

Preparation

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 dH2O to generate 10 mM L-Cysteine Standard solution. Aliquot and Store at -20°C. Avoid repeated freeze/thaw. Use within two months.

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 ddH2O. 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:
CYS Assay Buffer 193 µl
Diluted Enzyme Mix I 5 µl

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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)

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