Technical Bulletin

Cysteine Assay Kit

Catalogue number MAK255

Product Description

Cysteine (Cys) is a non-essential, sulfur containing amino acid in humans, related to cystine. Cysteine is important for protein synthesis, detoxification, and diverse metabolic functions. It is found in beta-keratin, which comprises the main protein in nails, skin, and hair. Cysteine is also important in collagen production, as well as skin elasticity and texture. A component of the antioxidant, glutathione, cysteine plays a role in the metabolism of essential biochemicals such as coenzyme A, heparin, and biotin. An elevated level of total cysteine can predict cardiovascular disease and metabolic syndromes.

The Cysteine Assay Kit detects the physiological concentration of cysteine in a variety of biological fluids. The assay is based on the cleavage of thiol group of reduced cysteine generating a fluorometric product (λex = 365 nm/ λem = 450 nm), 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. This high-throughput adaptable assay kit is simple and sensitive enough to detect to 10 µM of cysteine in a variety of samples.

This kit is suitable for use with serum, plasma, and other biological fluids.

Components

The kit is sufficient for 100 assays in 96-well plates.

- CYS Assay Buffer — 25 mL
  Catalogue Number MAK255A
- Enzyme Mix I — 50 µL
  Catalogue Number MAK255B
- Enzyme Mix II — 3 vls
  Catalogue Number MAK255C
- Reducing Agent — 2 vls
  Catalogue Number MAK255D
- HCY Blocker — 100 µL
  Catalogue Number MAK255E
- CYS Probe — 0.5 mL
  Catalogue Number MAK255F
- CYS Standard — 1 vl
  Catalogue Number MAK255G

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plates – It is recommended to use black plates for fluorescence assays.
- Fluorescence multiwell plate reader.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store all components at -20 °C, protected from light, is recommended.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

CYS Assay Buffer – Allow buffer to come to room temperature before use. Store at -20 °C.

CYS Probe – Warm to room temperature prior to use. Store at -20 °C. Protect from light.

Enzyme Mix I - Aliquot and store at -20 °C. Keep on ice during use and limit to two freeze/thaw cycles.

Enzyme Mix II - Reconstitute each vial in 1 mL of CYS Assay Buffer, as needed. Mix well by pipetting, store at 2–8 °C. Keep on ice during use. Use within 1 week of reconstitution.

Reducing Agent - Reconstitute each vial in 220 µL of CYS Assay Buffer, as needed. Mix well by pipetting, store at 2–8 °C. Keep on ice during use. Use within 1 week of reconstitution.

HCY Blocker - Allow to come to room temperature before use. Aliquot and store at -20 °C. Avoid repeated freeze/thaw cycles.

CYS Standard - Reconstitute in 1.26 mL of water, to generate a 10 mM L-Cysteine Standard solution. Mix well. Aliquot and store at -20 °C. Avoid repeated freeze/thaw cycles. Use within 2 months of reconstitution.

Procedure

All Samples and Standards should be run in duplicate.

CYS Standards for Fluorometric Detection

Dilute 10 µL of the 10 mM Cysteine Standard with 90 µL water to prepare a 1 mM Standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM Standard solution into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 n mole/well Standards. Add CYS Assay Buffer to each well to bring the volume to 10 µL.

Sample Preparation

Centrifuge biological fluids at 10,000 × g for 5 minutes at 2–8 °C. Collect the supernatant.

Add 5–10 µL of Samples into duplicate wells of a 96 well plate. Bring Samples to a final volume of 10 µL with CYS Assay Buffer.

To prepare a Sample background control, add 10 µL of CYS Assay Buffer to reaction well.

Assay Reaction

1. Dilute 2 µL of Enzyme Mix I to 18 µL with CYS Assay Buffer. 5 µL will be needed for each well to be assayed.
2. Set up the Master Reaction Mix according to the scheme in Table 1. 200 µL of the Master Reaction Mix is required for each reaction (well).

Table 1.
Master Reaction Mix

Reagent	Samples and Standards
CYS Assay Buffer	193 µL
Diluted Enzyme Mix I	5 µL
Reducing Agent	1 µL
HCY Blocker	1 µL

3. Mix well. Add 200 µL of the Master Reaction Mix to each Sample, Standard, and background control wells. Mix well using a multichannel pipette.
4. Incubate the plate for 30 minutes at 37 °C. Add 30 µL of Enzyme Mix II to each reaction/well and mix well using a multichannel pipette. Incubate for 5 minutes at 37 °C.

   Note: Incubation time for both Standard and the Sample wells must be consistent.

5. After incubation, add 5 µL of CYS Probe to each reaction/well. Mix well and measure fluorescence intensity (lex = 365/lem = 450 nm) in kinetic mode for at least 30 minutes at room temperature.
6. Measure the fluorescence in kinetic mode and choose two time points (T1 & T2) in the linear range (as short as 2 minutes) of the plot and obtain corresponding fluorescence reading for Samples and Standards. The Cysteine Standard curve can be read along with the Samples. It is important for the Samples and Standards to be read for the same length of time.

Results

The background is the value obtained for the 0 (assay blank) Cysteine Standard. Correct for the background by subtracting the 0 (assay blank) value from all readings. Background values can be significant and must be subtracted from all readings. Subtract the Sample Background Control value from the sample readings.

Note: A new standard curve must be set up each time the assay is run.

Use the values obtained from the appropriate Cysteine Standards to plot a standard curve. The amount of cysteine present in the sample may be determined from the standard curve.

Concentration of Cysteine:

Sa/Sv x DF = C

Sa = Amount of Cysteine in the unknown sample (nmole) from standard curve

Sv = Sample volume (µL) added into the wells

DF = Dilution Factor

C = Concentration of Cysteine in sample

Cysteine molecular weight: 121.16 g/mole

Sample Calculation:

Amount of Cysteine (Sa) = 5.84 nmole

(From standard curve)

Sample volume (Sv) = 50.0 µL

Concentration of Cysteine in sample:

5.84 nmole/50.0 µL × 1 = 0.117 nmole/µL

0.117 nmole/µL × 121.16 ng/nmole = 14.2 ng/µL

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