Cysteine Assay Kit

Cysteine Assay Kit is a detection kit for the quantification of Cysteine Content in tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82032

Package: 96 wells

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INTRODUCTION

Cysteine (symbol Cys or C) is a semiessential proteinogenic amino acid with the formula HOOC-CH-(NH2)-CH2-SH. The thiol side chain in cysteine often participates in enzymatic reactions as a nucleophile. The thiol is susceptible to oxidation to give the disulfide derivative cystine, which serves an important structural role in many proteins. When used as a food additive, it has the E number E920. It is encoded by the codons UGU and UGC. [Provide by Wikipedia: Cysteine]

PRINCIPLE OF THE ASSAY

This Cysteine Assay Kit is a simple colorimetric assay that measures the amount of Cysteine present in tissue extracts, cell lysate and cell culture supernatants. Cysteine concentration is determined by sodium tungstate dihydrate. The reaction products can be measured at a colorimetric readout at O.D. 600 nm. The concentration of Cysteine in the samples is then determined by comparing the O.D. 600 nm absorbance of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Component	Quantity	Storage information
Microplate	1 plate	RT
Assay Buffer	30 mL x 4 (ready to use)	4°C
Reaction Buffer	8 mL (ready to use)	4°C
Dye Reagent (lyophilized)	1 vial	4°C
Dye Reagent Diluent	10 mL (ready to use)	4°C
Standards (lyophilized)	1 vial	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 600 nm
- Centrifuge
- Mortar
- Deionized or Distilled water
- Ice
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and bacteria samples: Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mL of Assay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 10,000 x g for 10 minutes at 4°C, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue samples: Weigh out 0.1 g tissue, homogenize with 1 mL of Assay Buffer on ice. Centrifuged at 10,000 x g for 10 minutes at 4°C, and then take the supernatant into a new centrifuge tube and keep it on ice for detection.

Cell culture medium and other biological fluids: Add 0.9 mL of Assay Buffer into 0.1 mL of liquid sample, centrifuged at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly.

REAGENT PREPARATION

- Dye reagent: add 10 mL of Dye Reagent Diluent to dissolve before use, then put it in boiling water for 10 minutes.
- Standards: add 1 mL of distilled water to dissolve before use, the concentration will be 2 mol/L. Use the 2 mol/L Standards to prepare a series of standards according to the Table below.

Standard tube	Final standard conc. (mmol/L)	Volume of distilled water (μL)	Volume of 2 mol/L Standards (μL)
S1	2000	0	500
S2	1000	250	250 of S1
S3	500	250	250 of S2
S4	250	250	250 of S3
S5	125	250	250 of S4
S6	62.5	250	250 of S5
S7	31.3	250	250 of S6
S8	15.6	250	250 of S7
S9	7.8	250	250 of S8

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add **20 µL** of **samples, Blank (distilled water),** or serial **diluted Standards** into 96-well microplate.
2. Add **80 µL** of **Reaction Buffer** into each well and mix well.
3. Add **100 µL** of **Dye Reagent** into each well.
4. Mix well and incubate for **15 minutes** at **room temperature**.
5. Read the plate with a microplate reader at **O.D. 600 nm**.

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Blank and samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. According to the protein concentration of sample:

Cysteine (mmol/mg)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times C_{Protein})$

$= [2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / C_{Protein}$

6. According to the weight of sample:

Cysteine (mmol/g)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (V_{Sample} \times W / V_{Assay})$

$= [2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / W$

7. According to the quantity of cells or bacteria:

Cysteine ($mmol/10^4$)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / (N \times V_{Sample} / V_{Assay})$

$= [2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / N$

8. According to the volume of sample

Cysteine (mmol/mL)

$= [(C_{Standaard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})] / V_{Sample}$

$= 2 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

Note:

$C_{Protein}$: the protein concentration of sample, mg/mL;

W: the weight of sample, g;

$C_{Standard}$: the concentration of standard, 2 mol/L = 2 mmol/mL;

$V_{Standard}$: the volume of standard, 0.02 mL;

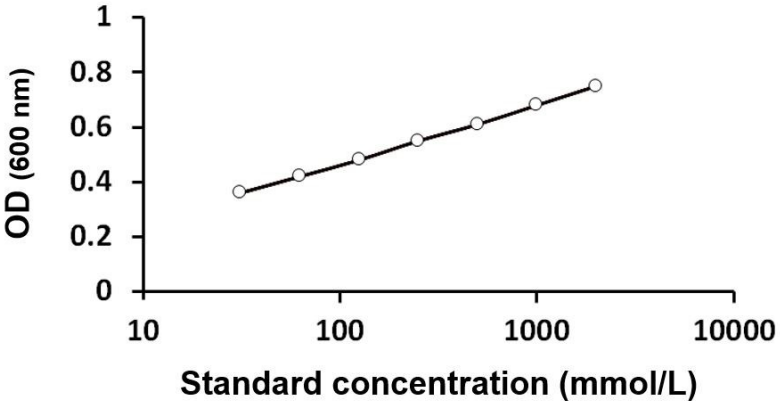
V_{Sample}: the volume of sample, 0.02 mL;

V_{Assay}: the volume of Assay buffer, 1 mL;

N: the quantity of cell or bacteria, $N \times 10^4$.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Cysteine Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

20 mmol/L