Glutathione Assay Kit

Catalog No.: abx096005

Size: 100 Assays

Storage: Store all components in the dark at 4°C.

Application: For quantitative detection of Glutathione concentration in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 0.01 mmol/L – 0.5 mmol/L

Introduction: Glutathione is tripeptide consisting of glycine, glutamic acid and cysteine amino acids. It functions as an antioxidant in living cells, protecting them from oxidative damage. It reacts with hydrogen peroxide in the presence of glutathione oxidase. Low concentrations of glutathione are associated with deficiencies in enzymes involved in glutathione metabolism, such as glucose-6-phosphate dehydrogenase, glutathione synthase and glutathione reductase.

Abbexa's Glutathione Assay Kit is designed to directly measure Glutathione concentrations in a variety of samples. In this assay, the concentration of the enzyme-catalyzed reaction product is directly proportional to the Glutathione concentration in the sample and can be calculated by measuring the absorbance at 412 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 8 ml
4. Dye Reagent: 1 vial
5. Diluent: 4 ml
6. Standard: 1 vial
7. Plate sealer: 3

Materials Required But Not Provided

1. Microplate reader (412 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**
  Add 4 ml of Diluent into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Ensure that the Dye Reagent has completely dissolved prior to use.

- **Standard Solution**
  Add 0.65 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 0.05 ml of this solution and add 0.95 ml of distilled water to prepare the Standard Solution (concentration 0.5 mmol/L). Unused Standard Solution can be stored in the dark at 4°C.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

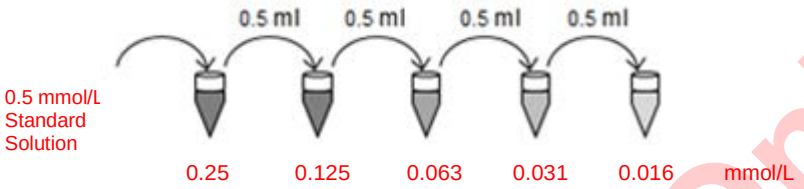
- **Liquid samples**
  Liquid samples can be used directly.

B. Assay Procedure

Warm all reagents to 37°C prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 5 tubes with 0.25 mmol/L, 0.125 mmol/L, 0.063 mmol/L, 0.031 mmol/L, and 0.016 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 0.5 mmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 80 µl of sample to the sample wells.
4. Add 80 µl of prepared standards to the standard wells.
5. Add 80 µl of distilled water to the blank wells.
6. Add 80 µl of Reaction Buffer to all wells.
7. Add 40 µl of Dye Reagent Solution to all wells.
8. Tap the plate gently to mix. Allow to stand for 10 minutes.
9. Read and record absorbance at 412 nm.

C. Calculations

Glutathione concentration per mg of protein:

$$\textbf{Glutathione}\ (\mu\text{mol/mg}) = \frac{C_{Standard} \times V_{Standard}}{V_{Sample} \times C_{Protein}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.5}{C_{Protein}} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Glutathione concentration per g of sample:

$$\textbf{Glutathione}\ (\mu\text{mol/g}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.5}{W} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

Glutathione concentration per 10^4 cells or bacteria:

$$\textbf{Glutathione}\ (\mu\text{mol}/10^4\ \textbf{cells}) = \frac{C_{Standard} \times V_{Standard} \times V_{Assay}}{V_{Sample} \times N} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} = \frac{0.5}{N} \times \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}}$$

where:

$C_{Protein}$	Concentration of protein (in mg/ml)
$C_{Standard}$	Concentration of highest standard (0.5 mmol/L = 0.5 µmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
V_{Assay}	Volume of Assay Buffer (1 ml)
V_{Sample}	Volume of sample (0.08 ml)
$V_{Standard}$	Volume of standard (0.08 ml)