Total Glutathione / Oxidized Glutathione (T-GSH / GSSG) Assay Kit

Catalog No.: abx294070

Size: 100 tests

Detection Range: 0.12 µmol/L T-GSH – 30 µmol/L T-GSH

Sensitivity: 0.12 µmol/L

Storage: Store the Assay Buffer, Chromogenic Reagent, Reagent Diluent, Scavenging Diluent, and Stop Solution at 4°C.
Store the Standard, Precipitating Reagent, Enzyme Solution, Scavenging Reagent, and the Substrate at -20°C.
Store the Chromogenic Reagent, Scavenging Reagent, and Substrate in the dark.

Application: For detection and quantification of Total Glutathione and Oxidized Glutathione content in serum, plasma, whole blood, red blood cell lysates, animal tissue homogenates, and cultured cell lysates.

Principle of the Assay

Abbexa's Total Glutathione / Oxidized Glutathione (T-GSH / GSSG) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Total (T-GSH) and Oxidized (GSSG) Glutathione content. The enzyme glutathione reductase can reduce GSSG to GSH, which is then oxidized back to GSSG by the chromogen DTNB. In this process, DTNB is reduced to the yellow compound TNB, with an absorbance maximum at 412 nm. The intensity of the color is proportional to the Total Glutathione content, which can then be calculated directly. Oxidized Glutathione content can be determined by first removing all non-oxidized Glutathione (GSH) content from the sample, then repeating the assay principle.

Kit components

1. Assay Buffer: 3 × 55 ml
2. Standard: 1 vial
3. Precipitating Reagent: 2 × 60 ml
4. Enzyme Solution: 1 vial
5. Chromogenic Reagent: 2 vials
6. Reagent Diluent: 3.6 ml
7. Scavenging Diluent: 1.4 ml
8. Scavenging Reagent: 0.2 ml
9. Substrate: 2 vials
10. Stop Solution: 50 ml

Materials required but not provided

1. Spectrophotometer (412 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Absolute (anhydrous) ethanol
5. Pipette and pipette tips
6. Microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum** and **Plasma:** Take 100 µl of serum or plasma into a fresh centrifuge tube, and add 400 µl Precipitating Reagent. Mix fully, using a vortex mixer for at least 30 seconds. Stand for 5 minutes at 4°C. Centrifuge at 3100 × g for 10 minutes. Carefully take the supernatant for detection, keep on ice, and analyze immediately.
- **Whole blood:** Collect blood using heparin or EDTA as the anticoagulant. Take 100 µl of blood into a fresh centrifuge tube, and add 400 µl Precipitating Reagent. Mix fully, using a vortex mixer for at least 30 seconds. Stand for 5 minutes at 4°C. Centrifuge at 3100 × g for 10 minutes. Carefully take the supernatant for detection, keep on ice, and analyze immediately.
- **Red blood cell lysates:** Collect blood using heparin or EDTA as the anticoagulant. Immediately centrifuge at 2000 rpm for 10 minutes, then carefully remove and discard the upper two liquid layers (the plasma and leukocytic buffy coat). Take 100 µl of the lower red blood cell layer into a fresh centrifuge tube and add 400 µl Precipitating Reagent. Mix fully, using a vortex mixer for at least 30 seconds. Stand for 5 minutes at 4°C. Centrifuge at 3100 × g for 10 minutes. Carefully take the supernatant for detection, keep on ice, and analyze immediately.
- **Tissue homogenates:** Carefully weigh out at least 20 mg of tissue, and wash in ice cold PBS (0.01 M, pH 7.4). Add the tissue into Precipitating Reagent in a ratio of 1 : 9, weight (mg) to volume (µl) (i.e. for 20 mg tissue, add 180 µl Precipitating Reagent). Homogenize manually, using a mechanical homogenizer or by ultrasonication, in an ice water bath at 4°C. Collect the resulting homogenate, and centrifuge at 10,000 × g for 10 minutes. Carefully take the supernatant, keep on ice, and analyze immediately.
- **Cultured cell lysates:** Count and collect at least 1 × 10^6 cells for analysis. Wash the cells with PBS (0.01 M, pH 7.4). Add the cells into Precipitating Reagent in a ratio of 1 : 400, cells (× 10^6) to volume (µl) (i.e. for 1 × 10^6 cells, add 400 µl Precipitating Reagent). Homogenize by ultrasonication in an ice water bath at 4°C. Centrifuge at 10,000 × g for 10 minutes at 4°C. Carefully take the supernatant, keep on ice, and analyze immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Precipitating Reagent, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
10% Rat heart tissue homogenate	10
10% Rat liver tissue homogenate	60
10% Rat brain tissue homogenate	10

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Stock (1 mmol/L GSSG) Standard Solution:** Dissolve the powdered Standard with 10 ml double-distilled water. Mix well, and ensure the powder has fully dissolved. The reconstituted Stock (1 mmol/L GSSG) Standard Solution can be stored for up to 1 month at -20°C.
- **Working (8 µmol/L GSSG) Standard Solution:** Prepare just enough Working (8 µmol/L GSSG) Standard Solution as required for the number of tubes tested. Prepare the solution by mixing Stock (1 mmol/L GSSG) Standard Solution with Precipitating Reagent in a ratio of 1 : 124 (i.e. to prepare 125 µl of Working (8 µmol/L GSSG) Standard Solution, mix fully 1 µl Stock (1 mmol/L GSSG) Standard Solution with 124 µl Precipitating Reagent). The resulting solution can be stored for up to 24 hours at 4°C. Prepare just before use.
- **Enzyme Working Solution:** Dilute 5 µl of Enzyme Solution with 95 µl Assay Buffer. Mix fully. The diluted Working Enzyme Solution can be stored for 4°C for up to 24 hours.
- **Chromogenic Working Solution**: Dissolve the Chromogenic Reagent with 1.5 ml of Reagent Diluent. Mix well, and ensure the powder has fully dissolved. The reconstituted Working Chromogenic Reagent can be stored for up to 3 months at -20°C.
- **Reaction Solution:** Prepare just enough Reaction Solution as required for the number of tubes tested. Prepare the solution by mixing Enzyme Working Solution, Chromogenic Working Solution, and Assay Buffer in a ratio of 1 : 1 : 25 (i.e. to prepare 810 µl of Reaction Solution, mix fully 30 µl Enzyme Working Solution, 30 µl Chromogenic Working Solution, and 750 µl Assay Buffer). The solution can be stored for up to 24 hours at 4°C. Prepare just before use.
- **Working Scavenging Diluent:** Prepare just enough Working Scavenging Diluent as required for the number of tubes tested. Prepare the solution by mixing Scavenging Diluent and double-distilled water in a ratio of 1:1 (i.e. to prepare 40 µl Working Scavenging Diluent, mix fully 20 µl Scavenging Diluent and 20 µl double-distilled water). The solution can be stored for up to 24 hours at 4°C. Prepare just before use.
- **Working Scavenging Reagent:** Prepare just enough Working Scavenging Reagent as required for the number of tubes tested. Prepare the solution by mixing Scavenging Reagent and absolute (anhydrous) ethanol in a ratio of 1 : 9 (i.e. to prepare 50 µl Working Scavenging Reagent, mix fully 5 µl Scavenging Reagent with 45 µl absolute (anhydrous) ethanol). The solution can be stored for up to 24 hours at 4°C. Prepare just before use.
- **Substrate Stock Solution:** Dissolve the Substrate powder with 150 µl double-distilled water. Mix well, until all the Substrate has fully dissolved. The reconstituted Substrate Stock Solution can be stored for up to 3 months at -70°C.
- **Substrate Working Solution:** Prepare just enough Substrate Working Solution as required for the number of tubes tested. Prepare the solution by mixing Substrate Stock Solution and Assay Buffer in a ratio of 1 : 79 (i.e. to prepare 400 µl Substrate Working Solution, mix fully 5 µl Substrate Stock Solution with 395 µl Assay Buffer). The solution can be stored for up to 24 hours at 4°C. Prepare just before use.

Note:

- The Scavenging Diluent is highly viscous. To ensure the correct volumes are taken up and dispensed, pipette the solution slowly and carefully.
- The Scavenging Reagent has a strong odor. Only open and handle this substance in a fume hood.
- Keep the Enzyme Solution on ice until it is used. Allow all other reagents to equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

Measurement of Total Glutathione (T-GSH)

1. Mark microcentrifuge tubes for each standard, sample, and blank. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 40 µl of Precipitating Reagent to the blank tube.
3. Add 40 µl of Working (8 µmol/L GSSG) Standard Solution to the standard tube.
4. Add 40 µl of sample to the sample tube.
5. Add 600 µl of Reaction Solution to all tubes.
6. Incubate all tubes at 25°C for 5 minutes.
7. Add 200 µl of Substrate Working Solution to all tubes. Vortex for 5 seconds to mix fully.
8. Incubate all tubes at 25°C for 25 minutes.
9. Add 400 µl of Stop Solution to each tube.
10. Transfer the solutions in each tubes to fresh cuvettes.
11. Zero the spectrophotometer with double-distilled water, then measure the OD values of each tube at 412 nm (0.5 cm path length).

Measurement of Oxidized Glutathione (GSSG)

12. Mark microcentrifuge tubes for each standard, sample, and blank. *It is strongly recommended to prepare all the tubes in duplicate.*
13. Add 20 µl of Working Scavenging Diluent to all tubes.
14. Add 100 µl of Precipitating Reagent to the blank tube. Vortex to mix fully.
15. Add 100 µl of Working (8 µmol/L GSSG) Standard Solution to the standard tube. Vortex to mix fully.
16. Add 100 µl of sample to the sample tubes. Vortex to mix fully.
17. From each tube, take 100 µl of the resulting solution and transfer to fresh tubes. Label these tubes appropriately as standard, sample, or blank.
18. Add 4 µl of Working Scavenging Reagent to all tubes. Immediately vortex to mix fully, then incubate at 25°C for 1 hour.
19. From each tube, take 40 µl of the incubated solution and transfer to a fresh tubes. Label these tubes appropriately as standard, sample, or blank.
20. Add 600 µl of Reaction Solution to all tubes.

21. Incubate all tubes at 25°C for 5 minutes.
22. Add 200 µl of Substrate Working Solution to all tubes. Vortex for 5 seconds to mix fully.
23. Incubate all tubes at 25°C for 25 minutes.
24. Add 400 µl of Stop Solution to each tube.
25. Transfer the solutions in each tubes to fresh cuvettes.
26. Zero the spectrophotometer with double-distilled water, then measure the OD values of each tube at 412 nm (0.5 cm path length).

C. Calculation of Results

The amount of Total (T-GSH) and Oxidized (GSSG) Glutathione in each sample tested can be derived with the following formulae:

1. Serum, Plasma, Whole blood, and **Red blood cell samples:**

$$\text{T-GSH (µmol/L)} = \frac{(OD_{\text{Total Sample}} - OD_{\text{Total Blank}}) \times 16 \times 5 \times F_{\text{Total}}}{(OD_{\text{Total Standard}} - OD_{\text{Total Blank}})}$$

$$\text{GSSG (µmol/L)} = \frac{(OD_{\text{Oxidized Sample}} - OD_{\text{Oxidized Blank}}) \times 8 \times 5 \times F_{\text{Oxidized}}}{(OD_{\text{Oxidized Standard}} - OD_{\text{Oxidized Blank}})}$$

2. Tissue samples:

$$\text{T-GSH (µmol/kg)} = \frac{(OD_{\text{Total Sample}} - OD_{\text{Total Blank}}) \times 16 \times V_{\text{Precipitating Tissue}} \times F_{\text{Total Tisue}}}{(OD_{\text{Total Standard}} - OD_{\text{Total Blank}}) \times W}$$

$$\text{GSSG (µmol/kg)} = \frac{(OD_{\text{Oxidized Sample}} - OD_{\text{Oxidized Blank}}) \times 8 \times V_{\text{Precipitating Tissue}} \times F_{\text{Oxidized Tissue}}}{(OD_{\text{Oxidized Standard}} - OD_{\text{Oxidized Blank}}) \times W}$$

3. Cultured cell samples:

$$\text{T-GSH (µmol/}10^9\text{ cells)} = \frac{(OD_{\text{Total Sample}} - OD_{\text{Total Blank}}) \times 16 \times V_{\text{Precipitating Cell}} \times F_{\text{Total Cell}}}{(OD_{\text{Total Standard}} - OD_{\text{Total Blank}}) \times N}$$

$$\text{GSSG (µmol/}10^9\text{ cells)} = \frac{(OD_{\text{Oxidized Sample}} - OD_{\text{Oxidized Blank}}) \times 8 \times V_{\text{Precipitating Cell}} \times F_{\text{Oxidized Cell}}}{(OD_{\text{Oxidized Standard}} - OD_{\text{Oxidized Blank}}) \times N}$$

$$\text{Reduced GSH} = \text{T-GSH} - (2 \times \text{GSSG})$$

where:

$OD_{\text{Total Sample}}$	OD value of sample tested for T-GSH
$OD_{\text{Total Standard}}$	OD value of standard tested for T-GSH
$OD_{\text{Total Blank}}$	OD value of blank tested for T-GSH
F_{Total}	The dilution factor of sample tested for T-GSH
$OD_{\text{Oxidized Sample}}$	OD value of sample tested for GSSG
$OD_{\text{Oxidized Standard}}$	OD value of standard tested for GSSG
$OD_{\text{Oxidized Blank}}$	OD value of blank tested for GSSG
F_{Oxidized}	The dilution factor of sample tested for GSSG
$V_{\text{Precipitating Tissue}}$	The volume of Precipitating Reagent used to prepare the tissue sample
$F_{\text{Total Tissue}}$	The dilution factor of tissue sample tested for T-GSH
$F_{\text{Oxidized Tissue}}$	The dilution factor of tissue sample tested for GSSG
W	The fresh weight of the tissue sample
$V_{\text{Precipitating Cell}}$	The volume of Precipitating Reagent used to prepare the cell sample
$F_{\text{Total Cell}}$	The dilution factor of cell sample tested for T-GSH
$F_{\text{Oxidized Cell}}$	The dilution factor of cell sample tested for GSSG
N	The factor of the number of cells tested compared to 1×10^6 This can be calculated with the formula $\frac{\text{Number of cells in tested sample}}{1 \times 10^6}$

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.