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Product Information

Reduced Glutathione (GSH) Assay Kit

Catalog Number **MAK364**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Glutathione (GSH), a thiol-containing tripeptide (γ-glutamyl-cysteinyl-glycine), is a key antioxidant in many species. It has been highly implicated in the detoxification/elimination of xenobiotics (naturally occurring harmful compounds such as free radicals, hydroperoxides etc.) and in the maintenance of the oxidation state of protein sulfhydryl groups. In addition, GSH plays a pivotal role in the pathogenesis of numerous human diseases including cancer and cardiovascular diseases. Glutathione is present in cells in both reduced (GSH) and oxidized (GSSG) forms with GSH being the predominant species under normal physiological conditions inside cells. Thus, pathologic conditions causing oxidative stress would result in increased levels of GSSG. Therefore, the measurement of intracellular GSH appears to be a sensitive indicator of the overall cell health, and its ability to resist toxic challenges.

The Reduced Glutathione (GSH) Assay Kit is based on an enzymatic cycling method in the presence of GSH and a chromophore. The reduction of the chromophore produces a stable product, which can be followed by kinetically measuring the absorbance at 450 nm (A_{450}). Therefore, the absorbance is directly proportional to the amount of GSH in the sample. The kit is highly specific and sensitive because GSSG does not interfere with the assay. The kit includes 5-Sulfosalicylic acid (SSA) to protect the GSH endogenous content of the samples. (SSA acts as a deproteinizing and antioxidant agent.) The assay is simple, reproducible, and can specifically detect as low as 50 pmol/well of reduced Glutathione (GSH) in a 100 µL reaction.

The kit is suitable for the measurement of reduced glutathione in various biological samples/preparations including tissue homogenates (liver, etc.) and cell lysates (Hep G2, Jurkat, etc.).

Components

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

GSH Assay Buffer Catalog Number MAK364A	50 mL
Substrate Mix A Catalog Number MAK364B	1 mL
Substrate Mix B Catalog Number MAK364C	1 vial
Enzyme Mix A Catalog Number MAK364D	15 µL
Enzyme Mix B Catalog Number MAK364E	120 µL
Enzyme Mix C Catalog Number MAK364F	1 vial
Sulfosalicylic Acid (SSA, 1 gram) Catalog Number MAK364G	1 each
GSH Standard Catalog Number MAK364H	1 vial

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Clear 96-well plates
- Refrigerated microcentrifuge capable of RCF ≥12,000 × *g*

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 components at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Preparation Instructions
Reagent Preparation
GSH Assay Buffer: may be stored at either 4 °C or –20 °C. Warm to room temperature prior to use.

Substrate Mix A, Enzyme Mix A, and Enzyme Mix B: Ready for use, keep on ice during assay.

Substrate Mix B: Reconstitute with 220 µL of GSH Assay Buffer and mix thoroughly.

Enzyme Mix C: Dissolve in 220 µL of GSH Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at –20 °C. Use within two months once reconstituted.

Sulfosalicylic Acid (SSA, 1 gram): Wear gloves while handling SSA. Add 19 mL of ultrapure water to make a 5% solution. Store at 4 °C, stable for 6 months once 5% solution is prepared.

GSH Standard: Reconstitute vial with 65 µL of ultrapure water to generate a 50 nmol/µL GSH Standard Solution. Once reconstituted, standard is stable for 2 months when stored at –20 °C.

Procedure
Sample Preparation
Notes:
- GSH is extremely labile: it is suggested to normalize data by protein content. Prepare two parallel sample homogenates from the same sample (the second one using the GSH Assay Buffer). Use the second replicate for protein measurement.
- Sample Preparation is critical for accurate determination of glutathione. It is suggested to use fresh, perfused samples and/or recently collected cells. If the assay cannot be performed immediately, extracts may be stored at –70 °C for 5 days.

1. Rapidly homogenize 100 mg of tissue or 100 µL of pelleted cells with 100 µL of 5% SSA Solution. GSH is sensitive to oxidation and/or degradation during sample preparation, acidification of samples with SSA should be done as quickly as possible to minimize autoxidation and degradation.
2. Vortex vigorously and keep on ice for 10 minutes.
3. Centrifuge samples at 12,000 × *g* at 4 °C for 20 minutes.
4. Collect the supernatant and keep on ice.
5. Dilute samples 5 to 20-fold with GSH Assay Buffer.
6. For Sample wells, add 2–10 µL of diluted sample(s) to designated well(s) of a clear 96 well plate. It is suggested to use 3–5 different amounts of each sample per well to ensure the readings are within the standard curve range and the signal kinetics are within the lineal range.
7. For the Sample Background Control, add the same volume of diluted samples to designated well(s).
8. Adjust the volume of Sample and Sample Background Control wells to 20 µL with GSH Assay Buffer.

Standard Curve Preparation
Prepare a 0.2 nmol/µL premix by diluting 2 µL of the 50 nmol/µL GSH Standard with 498 µL of GSH Assay Buffer, mix well. Prepare GSH Standards in desired wells of a clear 96 well plate according to Table 1.

Table 1.
Preparation of GSH Standards

Well	0.2 nmol/µL Premix	GSH Assay Buffer	GSH (nmol/well)
1	0 µL	20 µL	0
2	2 µL	18 µL	0.4
3	4 µL	16 µL	0.8
4	6 µL	14 µL	1.2
5	8 µL	12 µL	1.6
6	10 µL	10 µL	2

Reaction Mix
1. Prepare a 100-fold Dilution of Enzyme Mix A (i.e. Dilute 2 µL of Enzyme Mix A stock solution with 198 µL of GSH Assay Buffer), mix well and keep on ice. Prepare dilution fresh as needed, do not store.
2. Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 80 µL of Reaction Mix according to Table 2, mix well.

Table 2.
Preparation of Reaction Mixes

Reagent	Sample Reaction Mix	Sample Background Control Reaction Mix
Substrate Mix A	10 µL	10 µL
Diluted Enzyme Mix A	10 µL	–
Enzyme Mix B	1 µL	1 µL
Enzyme Mix C	2 µL	2 µL
Substrate Mix B	2 µL	2 µL
GSH Assay Buffer	55 µL	65 µL

3. Add 80 µL of the Sample Reaction Mix to each well containing the GSH Standard and Sample(s). Add 80 µL of the Sample Background Control Reaction Mix to well(s) containing Sample Background Control.

Measurement
Measure absorbance at 450 nm (A_{450}) in kinetic mode at room temperature for 40–60 minutes. Choose two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding absorbance values ($A_{450(1)}$ and $A_{450(2)}$).

Results
1. Calculate the rate of each Standard Reading:

   $$\text{Rate} = [\Delta A_{450}\ (A_{450(2)} - A_{450(1)})]/[\ (\Delta t\ (t_2 - t_1)]$$

2. Subtract 0 Standard Rate from all Standards Rates.
3. Plot the GSH Standard Curve Rate (A_{450}/min) vs. GSH (nmol/well) and obtain the slope of the curve.
4. Calculate the Rate of the Background Corrected Samples:

   $$(\Delta A_{450\text{Sample Background Control}}/\Delta t_{\text{ Sample Background Control}}) - (\Delta A_{450\text{Sample}}/\Delta t_{\text{ Sample}})$$

5. Apply the Rate of the Background Corrected Samples to GSH Standard Curve to obtain the corresponding amounts of GSH in samples (B)

   $$B = \frac{(\text{Rate}_{\text{Sample}} - \text{Rate}_{\text{Sample Background Control}})}{\text{Slope of standard curve}}$$

6. GSH amount in sample (nmol/mg) =

   $$\frac{B}{(V \times P)} \times D$$

where:
B = GSH from Standard Curve (nmol)
V = Sample volume added into reaction well (mL)
P = Sample Concentration in mg protein/mL
D= Sample dilution factor

Figure 1.
Typical A_{450} from GSH Standard Curve

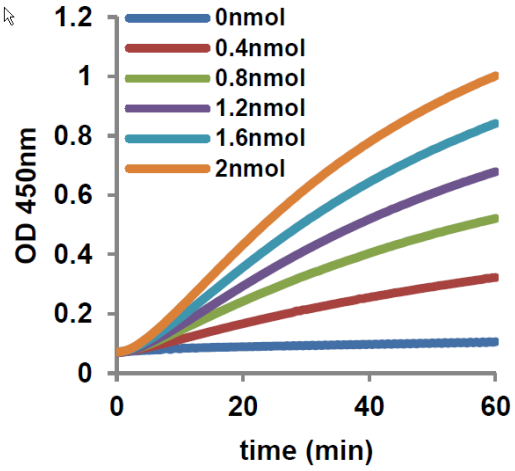


Figure 2.
Typical GSH Standard Curve (Rate)

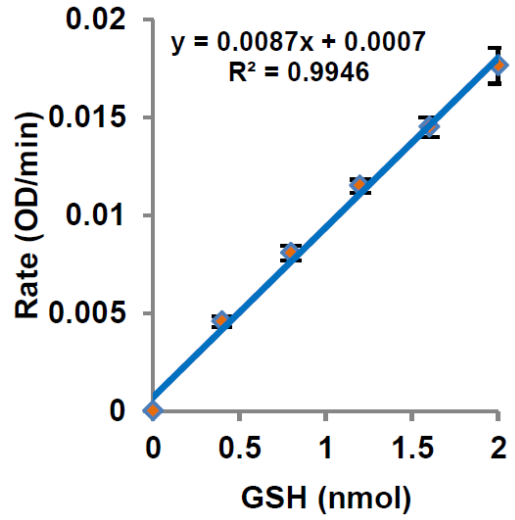
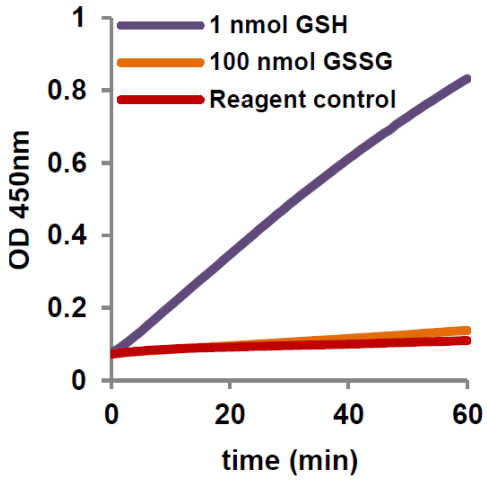
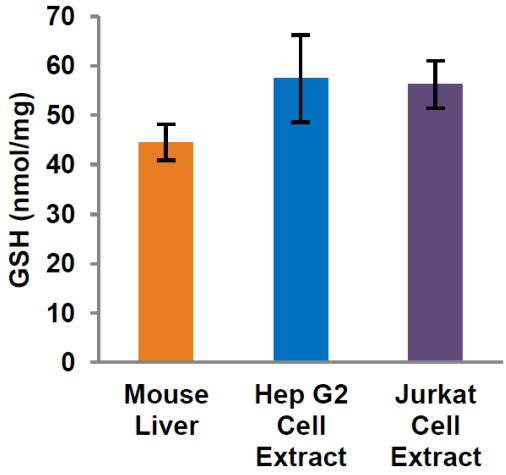


Figure 3.
Assay Specificity



Measurement of GSH (1 nmol) and GSSG (100 nmol). The assay kit can effectively discriminate between reduced GSH and oxidized GSSG forms.

Figure 4.
Measurement of GSH



Measurement of GSH in Mouse Liver (10 µg protein), Hep G2 Cell Extract (6 µg protein) and Jurkat Cell Extract (10 µg protein). All assays were performed following kit procedure.

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