Reduced Glutathione (GSH) Assay Kit (Colorimetric)

(Catalog # BN00700; 100 assays; Store at -20°C)

I. Introduction:

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 cardio-vascular diseases. Glutathione is present in cells in both reduced (GSH) and oxidized (GSSG) forms- 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 of resisting toxic challenges. Assay Genie's Reduced Glutathione 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 kinetically at 450 nm. Therefore, its absorbance is directly proportional to the amount of GSH in the sample. Our kit is the most specific and sensitive quantitative assay in the market because GSSG does not interfere with the assay. The kit includes 5-Sulfosalicylic acid (SSA) in order to protect samples' GSH endogenous content. (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 form of Glutathione (GSH) in a 100 µl reaction.

II. Applications:

- Measurement of Reduced Glutathione in various biological samples/preparations

III. Sample Type:

- Tissue Homogenates: Liver, etc.
- Cell Lysates: Hep G2, Jurkat, etc.

IV. Kit Contents:

Components	BN00700	Cap Code	Part Number
GSH Assay Buffer	50 ml	NM	BN00700-1
Substrate Mix A	1.0 ml	Brown	BN00700-2
Substrate Mix B	1 vial	Red	BN00700-3
Enzyme Mix A	15 µl	Violet	BN00700-4
Enzyme Mix B	120 µl	Orange	BN00700-5
Enzyme Mix C	1 vial	Green	BN00700-6
Sulfosalicylic Acid (SSA, 1 gram)	1 bottle	WM	BN00700-7
GSH Standard	1 vial	Yellow	BN00700-8

V. User Supplied Reagents and Equipment:

- Microplate reader capable of absorbance measurement
- 96-well clear plate
- Dounce Tissue Homogenizer

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **GSH Assay Buffer:** Store at either 4 ºC or -20 ºC. Bring to room temperature before use.
- **Substrate Mix A:** Ready for use, store at -20 °C, use on ice.
- **Substrate Mix B:** Reconstitute with 220 µl of GSH Assay Buffer and mix thoroughly. Store at -20 °C.
- **Enzyme Mix A:** Ready for use, store at -20 °C, use on ice.
- **Enzyme Mix B:** Ready for use, store at -20 °C, use on ice.
- **Enzyme Mix C:** Dissolve in 220 µl GSH Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20 °C. Use within two months.
- **Sulfosalicylic Acid (Wear gloves while handling SSA):** Add 19 ml of **dH_2O** to make 5% solution. Store at 4 °C, stable for 6 months.
- **GSH Standard:** Dissolve in 65 µl **dH_2O** to generate 50 nmol/µl GSH Standard Solution. Store at -20 ºC, stable for 2 months.

VII. Reduced Glutathione Assay Protocol:

1. Sample Preparation:

Rapidly homogenize tissue (100 mg) or 100 µl of pelleted cells with 100 µl of 5% SSA Solution. Vortex vigorously and keep on ice for 10 min. Centrifuge samples at 12,000 x *g* at 4 ºC for 20 min. Collect the supernatant and keep on ice. Dilute samples **5-20 fold** with GSH Assay Buffer. **Sample well**: add 2-10 µl of **diluted samples** to wells of a clear 96-well plate. **Sample Background Control:** Add same volume of diluted samples to designated well(s). Adjust the volume of Sample and Sample Background Control to **20 µl/well** with GSH Assay Buffer.

Note:

a. GSH is extremely labile: If you want to normalize your 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.

b. Sample Preparation is critical for accurate determination of glutathione. We recommend using fresh, perfused samples and/or recently collected cells. If the assay cannot be performed immediately, extracts may be stored at -70 ºC for 5 days

c. 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.

d. We suggest using 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.

2. **Standard Curve Preparation:** Dilute the 50 nmol/µl GSH Standard to 0.2 nmol/µl by adding 2 µl of the Standard to 498 µl of GSH Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl of Diluted GSH Standard into a series of wells. Adjust volume to 20 µl/well with GSH Assay Buffer to generate 0, 0.4, 0.8, 1.2, 1.6, 2 nmol/well of the GSH Standard.

3. **Reaction Mix Preparation:** Prepare a 100-fold Dilution of Enzyme Mix A (i.e. Dilute 2 µl of Enzyme Mix A stock solution with 198 µl GSH Assay Buffer), mix well and keep on ice. Mix enough reagents for the number of assays to be performed. For each well, prepare a total 80 µl Reaction Mix containing the following components. Mix well before use:

	Sample Reaction Mix	Sample Background Control 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

Add 80 µl of the Reaction Mix to each well containing the GSH Standard, Sample(s); Add 80 µl of Sample Reagent Control Mix to well(s) containing Sample Background Control.

Note: Do not store the Diluted Enzyme Mix A. Prepare fresh dilutions as needed.

4. **Measurement:** Measure absorbance (OD= 450 nm) in kinetic mode at room temperature for 40-60 min. Choose two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding absorbance values (OD_1 and OD_2).

5. **Calculation:** Calculate the rate of each Standard Reading: **Rate= [ΔOD (OD_2-OD_1)]/[(Δt (t_2 - t_1))]** (Fig a). Subtract 0 Standard Rate from all Standards Rates. Plot the GSH Standard Curve Rate (OD/min) vs. GSH (nmol/well) and obtain the slope of the curve (Fig b). Calculate the Rate of the Background Corrected Samples by subtracting the Sample Background Control **Rate (ΔOD/Δt)** from Sample **Rate (ΔOD/Δt)**. Apply the Rate of the Background Corrected Samples to GSH Standard Curve to obtain the corresponding amounts of GSH in samples (B=[$Rate_{sample}$ – $Rate_{Sample\ Background\ Control}$]/the slope of standard curve).

GSH amount in sample = (B/ V*P) x D = nmol/mg

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

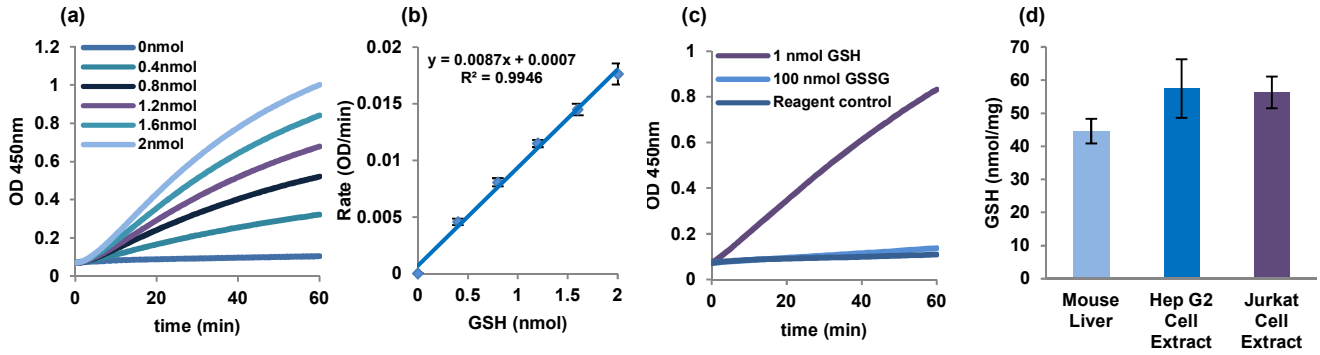

Figure: (a-b) GSH Standard Curve, results from multiple experiments. (c) Assay Specificity. Measurement of GSH (1 nmol) and GSSG (100 nmol). The assay kit can effectively discriminate between reduced GSH and oxidized GSSG forms. (d) Measurement of GSH in Mouse Liver (10 µg protein), Hep G2 Cell Extract (6 µg protein) and Jurkat Cell Extrac (10 µg protein). All assays were performed following kit protocol.

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