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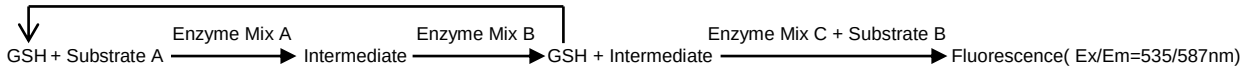
PicoProbe™ Reduced Glutathione (GSH) Assay Kit (Fluorometric)

09/17

(Catalog # K740-100; 100 assays; Store at -20°C)

I. Introduction:

Glutathione (GSH), a thiol-containing tripeptide, is a key antioxidant in living systems. Intracellular GSH status appears to be a sensitive indicator of the overall health of a cell. BioVision's PicoProbe™ Reduced Glutathione Assay Kit is based on a specific enzymatic cycling method in the presence of GSH and a fluorophore. The reduction of the fluorophore produces a stable fluorescent product, the fluorescence of which is directly proportional to the amount of GSH in the sample and can be followed kinetically (Ex/Em=535/587 nm). Our assay is the most specific and sensitive in the market. Oxidized Glutathione does not interfere with the assay. The assay is simple, reproducible and can specifically detect as low as 8 pmol/well of reduced form of Glutathione (GSH) in a 100 µl reaction.



II. Applications:

- Measurement of Glutathione in various biological samples/preparations

III. Sample Type:

- Tissue Homogenates and Cell Lysates: Liver, Hep G2, Jurkat, etc.
- Biological Fluids: Plasma, etc.

IV. Kit Contents:

Components	K740-100	Cap Code	Part Number
GSH Assay Buffer	50 ml	NM	K740-100-1
PicoProbe™ (in DMSO)	0.4 ml	Blue	K740-100-2
Substrate Mix A	1.0 ml	Brown	K740-100-3
Substrate Mix B	1 vial	Red	K740-100-4
Enzyme Mix A	15 µl	Violet	K740-100-5
Enzyme Mix B	120 µl	Orange	K740-100-6
Enzyme Mix C	1 vial	Green	K740-100-7
Sulfosalicylic Acid (SSA, 1 gram)	1 bottle	WM	K740-100-8
GSH Standard	1 vial	Yellow	K740-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of fluorescence measurement
- 96-well white plate with flat bottom
- Dounce Tissue Homogenizer (Cat. #1998 or its equivalent)

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.
- **PicoProbe™:** Ready to use as supplied. Warm to room temperature before use. Store at -20 °C.
- **Substrate Mix A, Enzyme Mix A, and Enzyme Mix B:** 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 C:** Dissolve in 220 µl GSH Assay Buffer and mix thoroughly. Store at -20 °C and 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:

Tissue and Cell Extracts: Rapidly homogenize (Cat. #1998 or equivalent) tissue (100 mg) or 100 µl (>10^7) of pelleted cells with 300 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 **20-40 fold** with GSH Assay Buffer. **Sample well**: add 2-10 µl of **diluted samples** to wells of a white 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

Plasma: Plasma has low concentrations of GSH (2-20 µmol/L). We recommend spiking samples with a known amount of GSH Standard and run parallel unspiked samples. **GSH spike:** Dilute 50 nmol/µl of GSH to 1 nmol/µl by adding 2 µl of the GSH Standard to 98 µl GSH Assay Buffer; further dilute to 20 pmol/µl of GSH by adding 8 µl of 1 nmol/µl GSH to 392 µl of GSH Assay Buffer. **Spiked Sample**: 100 µl plasma + 100 µl of 20 pmol/µl GSH + 200 µl of 5% SSA.; **Unspiked Sample**: 100 µl plasma +100 µl of GSH Assay Buffer + 200 µl of 5% SSA; **Background Control**: 100 µl of dH_2O + 100 µl of GSH Assay Buffer + 200 µl of 5% SSA. Vortex each sample and leave for 10 min on ice. Centrifuge at 12,000 x g at 4 ºC for 10 min., collect the supernatant and keep on ice. Add 10 µl of **Spiked Sample**, **Unspiked Sample** or **Background Control** to wells of a 96-well white plate. *The GSH Standard spiked in sample is 50 pmol*. Adjust the volume of Spiked Sample, Unspiked Sample and Background Control to **20 µl/well** with GSH Assay Buffer.

Notes:

a. 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. GSH is sensitive to oxidation: acidification of samples with SSA should be carried out as quickly as possible

2. **Standard Curve Preparation:** Dilute the 50 nmol/µl GSH Standard to 1 nmol/µl by adding 2 µl of the Standard to 98 µl of GSH Assay Buffer and mix well. Further dilute the 1 nmol/µl GSH Standard to 10 pmol/µl by adding 2 µl of the 1 nmol/µl of the Standard to 198 µl of GSH Assay Buffer. Add 0, 2, 4, 6, 8, 10 µl of 10 pmol/µl GSH Standard into a series of wells. Adjust volume to 20 µl/well with GSH Assay Buffer to generate 0, 20, 40, 60, 80,100 pmol/well of 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 Mix containing the following components. Mix well before use:

	Reaction Mix	Background 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
PicoProbe™	1 µl	1 µl
GSH Assay Buffer	54 µl	64 µl

Add 80 µl of the Reaction Mix to each well containing the GSH Standard, Sample(s); add 80 µl of Background 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 fluorescence (Ex/Em = 535/587 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 fluorescence values (RFU_1 and RFU_2).
5. **Calculation:** Calculate the rate of each Standard Reading: **Rate= [ΔRFU (RFU_2-RFU_1)]/[(Δt (t_2 - t_1)]**. Subtract 0 Standard Rate from all Standards' Rates. Plot the GSH Standard Curve Rate (RFU/min) vs. GSH (pmol/well) and obtain the slope of the curve (Fig a). Calculate the Rate of the Background Corrected Samples by subtracting the **Sample Background Control Rate (ΔRFU/Δt)** from **Sample Rate (ΔRFU/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]).

$$\textbf{GSH amount in spiked sample (B)} = \left(\frac{(\text{Rate}(\text{unspiked samples})\ (\text{corrected}))}{(\text{Rate}(\text{spiked samples})\ (\text{corrected}))\text{-}(\text{Rate}\ (\text{unspiked samples})\ (\text{corrected}))}\right) * 50\ pmol$$

Where: 50 pmol is GSH Standard spiked in samples

GSH amounts in sample = (B/ V*P) x D = pmol/ µg =nmol/mg

Where: **B** = GSH from Standard Curve (pmol)
V = the Sample volume added into reaction well (µl)
P = Initial Sample Concentration in µg-protein/ µl
D = Sample Dilution Factor

GSH concentration in plasma can be expresses in µM.

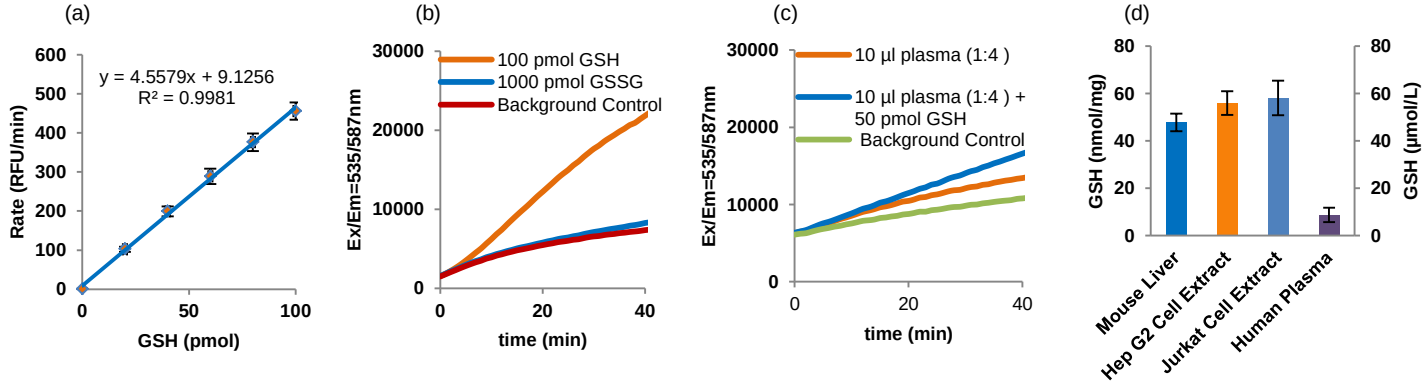


Figure: (a) GSH Standard Curve, results from multiple experiments. (b) Assay Specificity: measurement of GSH (100 pmol) and GSSG (1000 pmol). The kit can effectively discriminate reduced GSH and oxidized GSSG forms. (c) Measurement of GSH in Human Plasma (Spiked and Unspiked samples) (d) Measurement of GSH in Mouse Liver (1.2 µg protein), HepG2 Cell Extract (0.6 µg protein), Jurkat Cell Extract (1 µg protein) and Human Plasma (10 µl, 1:4 dilution). All assays were performed following kit protocol.

VIII. RELATED PRODUCTS:

Glutathione (GSH/GSSG/Total) Fluorometric Assay Kit (K264)
Reduced Glutathione Kit (Colorimetric) (K464)
Dounce Tissue Homogenizer (1998)
Glutathione Colorimetric Assay Kit (K261)

FOR RESEARCH USE ONLY! Not to be used on humans