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ab239727
GSH Assay Kit (Colorimetric)

For the detection of reduced glutathione in various biological samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

GSH Assay Kit (Colorimetric) (ab239727) provides a simple in vitro assay for detecting reduced GSH levels in apoptosis and other pathological processes. The 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.

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.

2. Protocol Summary

Prepare samples, reagents and standard curves.

Add 2-10 µl of diluted samples to wells of a clear 96-well plate.

Adjust volume to 20 µl/well with GSH Assay Buffer.

Prepare a 100-fold dilution of Enzyme Mix A and keep on ice.

Add 80 µl of the Reaction Mix to standard and sample wells.

Measure absorbance (OD=450 nm) in kinetic mode at room temperature fro 40-60 min.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
GSH Assay Buffer	50 mL	-20°C
Substrate Mix A	1.1 mL	-20°C
Developer Solution III/Substrate Mix B	1 vial	-20°C
Enzyme Mix A	15 µl	-20°C
Enzyme Mix B	120 µl	-20°C
Enzyme Mix V/Enzyme Mix C	1 vial	-20°C
Sulfosalicylic Acid (SSA, 1 gram)	1 unit	-20°C
GSH Standard	1 vial	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 96-well plate with flat bottom.
- Microplate reader capable of absorbance measurement.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 GSH Assay Buffer:

Store at -20°C or 4°C. Bring to room temperature (RT) before use.

6.2 Substrate Mix A:

Ready to use as supplied. Store at -20°C, use on ice.

6.3 Developer Solution III/Substrate Mix B:

Reconstitute with 220 µL of GSH Assay Buffer and mix thoroughly. Store at -20°C.

6.4 Enzyme Mix A:

Ready to use as supplied. Store at -20°C, use on ice.

6.5 Enzyme Mix B:

Ready to use as supplied. Store at -20°C, use on ice.

6.6 Enzyme Mix V/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 2 months.

6.7 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.

6.8 GSH Standard:

Dissolve in 65 µL dH_2O to generate 50 nmol/µL GSH Standard Solution. Store at -20°C, stable for 2 months.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Dilute the 50 nmol/µl GSH Standard to 0.2 nmol/µl by adding 2 µl of the Standard to 498 µl of the GSH Assay Buffer, mix well.

7.2 Add 0, 2, 4, 6, 8, and 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 and 2 nmol/well of the GSH Standard.

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

8. Sample Preparation

8.1 Rapidly homogenize tissue (100 mg) or 100 µl of pelleted cells with 100 µl of 5% Sulfosalicylic Acid/SSA Solution. Vortex vigorously and keep on ice for 10 min.

8.2 Centrifuge samples at 12,000 x *g* at 4 °C for 20 min. Collect the supernatant and keep on ice.

8.3 Dilute samples 5-20 fold with GSH Assay Buffer.

8.4 **Sample well:** Add 2-10 µl of diluted samples to wells of a clear 96-well plate.

8.5 **Sample Background Control**: Add same volume of diluted samples to designated well(s).

8.6 Adjust the volume of Sample and Sample Background Control to 20 µl/well with GSH Assay Buffer.

Δ 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 °C for 5 days

c. GSH is sensitive to oxidation and/or degradation during sample preparation, acidification of samples with Sulfosalicylic Acid/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.

9. Assay Procedure

9.1 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.

9.2 Prepare 80 µL Reaction Mix for each well to be assayed as per the table and mix well. Add 80 µL of Reaction Mix into each well containing the GSH Standard and sample wells. Add 80 µL of Sample Reagent Control Mix to well(s) containing Sample Background Control. Mix well before use.

	Reaction Mix	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 V /Enzyme Mix C	2 µL	2 µL
Developer Solution III /Substrate Mix B	2 µL	2 µL
GSH Assay Buffer	55 µL	65 µL

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

Measure absorbance at 450 nm in a 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).

10. Data Analysis

10.1 Calculate the rate of each Standard Reading: Rate= [ΔOD (OD_2-OD_1)]/[(Δt (t_2 - t_1)] (Fig 1).

10.2 Subtract 0 Standard Rate from all Standard Rates. Plot the GSH Standard Curve Rate (OD/min) vs. GSH (nmol/well) and obtain the slope of the curve (Fig 2).

10.3 Calculate the Rate of the Background Corrected Samples by subtracting the Sample Background Control Rate (ΔOD/Δt) from Sample Rate (ΔOD/Δt).

10.4 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).

$$\text{GSH amount in sample} = (B/V*P) \times D = \text{nmol/mg}$$

Where: **B** is amount of GSH from Standard Curve (nmol)
V is sample volume added into the reaction well (mL)

P is sample concentration in mg-protein/ml

D is sample dilution factor

11. Typical Data

Typical data provided **for demonstration purposes only**.

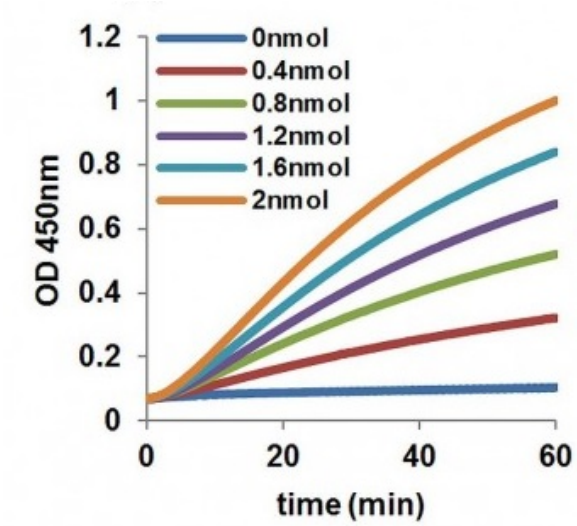

Figure 1. GSH Standard Curve, results from multiple experimets.

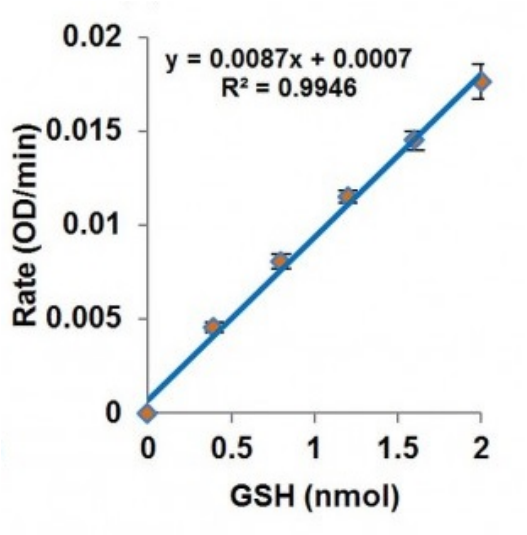

Figure 2. GSH Standard Curve, results from multiple experimets.

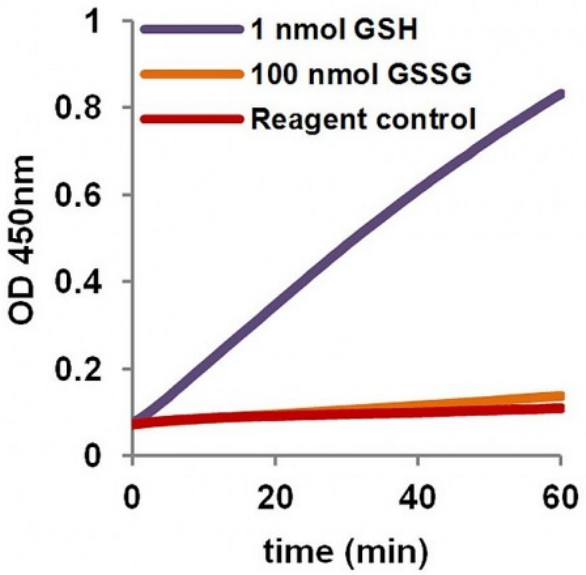

Figure 3. Assay Specificity. Measurement of GSH (1 nmol) and GSSG (100 nmol). The assay kit can effectively discriminate between reduced GSH and oxidized GSSG forms.

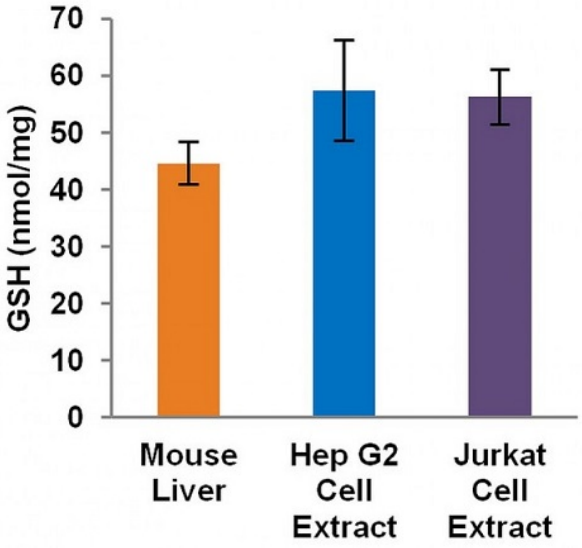

Figure 4. Measurement of GSH in Mouse Liver (10 µg protein), Hep G2 Cell Extract (6 µg protein) and Jurkat Cell Extrac (10 µg protein). All assays were performed following kit protocol.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)