Version 5, Last updated 8 April 2025

ab239709
GSH+GSSG / GSH Assay Kit (Colorimetric)

For the detection of GSH changes in apoptosis and other pathological processes.

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.

Table of Contents

1. Overview

GSH+GSSG / GSH Assay Kit (Colorimetric) (ab239709) provides a convenient, colorimetric method for analyzing either total glutathione or the reduced form glutathione alone using a microtiter plate reader.

The assay is based on the glutathione recycling system by DTNB and glutathione reductase. DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid which has a yellow color. Therefore, GSH concentration can be determined by measuring absorbance at 412 nm.

The generated GSSG can be reduced back to GSH by glutathione reductase, and GSH reacts with DTNB again to produce more 2-nitro-5-thiobenzoic acid. Therefore, the recycling system dramatically improves the sensitivity of total glutathione detection.

The kit includes the 5-Sulfosalicylic acid (SSA) for the removal of proteins from samples and for the protection of GSH oxidation and γ-glutamyl transpeptidase reaction.

The kit can quantify glutathione from 1-100 ng/well in a 200 µL reaction. For detecting lower glutathione concentrations, such as in blood samples, increasing reaction time will generate stronger signal.

The kit can also specifically detect the reduced form of glutathione (GSH) by omitting the glutathione reductase from the reaction mixture. The sensitivity for detecting the reduced form of glutathione (without recycling system) is 100 times lower than detecting the total glutathione.

2. Protocol Summary

Prepare samples and reagents.

Prepare standard curve.

Prepare substrate mix and add 160 µL to each well. Add 20 µL sample and standards.

Add 20 µL of Substrate Solution and incubate.

Read the absorbance at 405 nm or 415 nm using a microplate reader

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
Glutathione Reaction Buffer	100 mL	-20°C
DTNB/Glutathione Substrate (DTNB)	2 x 1 vial	-20°C
NADPH Generating Mix/NADPH Generating Mix (lyophilized)	2 x 1 vial	-20°C
Glutathione Reductase	2 x 25 µL	-20°C
Sulfosalicylic Acid/Sulfosalicylic Acid (SSA, 1 gram)	1 x 1 unit	-20°C
GSH Standard/GSH Standard (lyophilized, MW 307)	2 x 1 mg	-20°C

5. 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.

5.1 Glutathione Reaction Buffer:

Ready to use. Store at -20°C. Bring to room temperature before use.

5.2 DTNB/Glutathione Substrate (DTNB):

Add 1 mL of Glutathione Reaction Buffer to 1 vial of substrate and dissolve it completely. Store the remaining solution at –20°C, stable for 2 months.

5.3 NADPH Generating Mix/NADPH Generating Mix (lyophilized):

Add 1 mL of Glutathione Reaction Buffer to 1 vial. Store the solution at -20°C, stable for 2 months.

5.4 Glutathione Reductase:

Add 1 mL of Glutathione Reaction Buffer to 1 vial of the enzyme and dissolve. Use up the solution within 1 day.

5.5 Sulfosalicylic Acid/Sulfosalicylic Acid (SSA, 1 gram):

Add 19 mL of dH_2O to make 5% solution and then dilute 5 mL of the solution with Glutathione Reaction Buffer to make 1% SSA solution. Store at 4°C, stable for 6 months.

5.6 GSH Standard/GSH Standard (lyophilized, MW 307):

Add 1 mL of 1% SSA to the GSH standard vial to generate 1µg/µL GSH standard solution. Store at –20°C, stable for 2 months.

6. Standard Preparation

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

6.1 To generate standard curve for detecting the reduced form of glutathione only, add 50, 40, 30, 20, 10, and 0 µL of the 1 µg/µL GSH standard into each labeled microcentrifuge tubes, add 1% SSA to make up for a total volume of 100 µL/tube.

Standard #	1 µg/µL GSH standard (µL)	1% SSA (µL)	End amount in well (µg)
1	50	50	10
2	40	60	8
3	30	70	6
4	20	80	4
5	10	90	2
6	0	100	0

6.2 To generate standard curve for detecting the total glutathione, dilute the 1 µg/µL glutathione solution to 10 ng/µL with 1% SSA. Add 50, 40, 30, 20, 10, and 0 µl of the 10 ng/µL GSH standard into each labeled microcentrifuge tubes, add 1% SSA to make up for a total volume of 100 µL/tube.

Standard #	10 ng/µL GSH standard (µL)	1% SSA (µL)	End amount in well (ng)
1	50	50	100
2	40	60	80
3	30	70	60
4	20	80	40
5	10	90	20
6	0	100	0

7. Sample Preparation

– Peptide thiol may interfere with the assay of reduced form of glutathione. SSA treatment may not be able to completely remove all small peptides from samples. Further purification may be required to accurately measure the reduced form of glutathione. Peptide thiols don't significantly interfere with the total glutathione assay.

7.1 Cell Sample Preparation (0.5-1 x 10^6 cells/assay)

7.1.1 Treat cells by desired method. Concurrently incubate a control culture without treatment.

7.1.2 Collect cells by centrifugation at 700 x *g* for 5 min at 4°C. Remove supernatant.

7.1.3 Resuspend cell pellet in 0.5 mL ice-cold PBS. Transfer into a 1.5 mL microcentrifuge tube, and centrifuge at 700 x *g* for 5 min at 4°C. Remove supernatant.

7.1.4 Lyse cells in 80 µL ice-cold Glutathione Buffer. Incubate on ice for 10 min.

7.1.5 Add 20 µL of 5% SSA, mix well and centrifuge at 8000 x *g* for 10 min. Transfer supernatant to a fresh tube and use it for glutathione assay.

7.2 Tissue Sample Preparation (100 mg)

7.2.1 Homogenize the tissue in 0.4 mL of Glutathione Buffer.

7.2.2 Add 100 µL of 5% SSA, mix well, and centrifuge at 8000 x *g* for 10 min.

7.2.3 Transfer supernatant to a fresh tube and use it for glutathione assay.

7.3 Plasma Sample Preparation

7.3.1 Centrifuge anticoagulant treated blood at 1000 x *g* for 10 min at 4°C.

7.3.2 Transfer the top plasma layer to a new tube and add 1/4 vol of 5 % SSA. Mix well.

7.3.3 Centrifuge at 8000 x *g* for 10 min at 4°C.

7.3.4 Transfer supernatant to a new tube, and use it for the glutathione assay.

7.4 Erythrocyte Sample Preparation

7.4.1 Centrifuge anticoagulant treated blood at 1000 x *g* for 10 min at 4°C.

7.4.2 Discard the supernatant and the white buffy layer.

7.4.3 Lyse the erythrocytes with 4 vol of Glutathione Buffer. Keep on ice for 10 min.

7.4.4 Add 1 vol 5% SSA, mix well, and centrifuge at 8000 x *g* for 10 minutes. Transfer supernatant to a fresh tube and use it for glutathione assay.

Δ Note: Erythrocytes can be isolated from the remaining sample solution after the plasma sample isolation.

8. Assay Procedure

8.1 Prepare enough Reaction Mix for the standard and samples to be assayed in a 96-well plate.

	Reaction Mix
NADPH Generating Mix	20 µL
Glutathione Reductase	20 µL
Glutathione Reaction Buffer	120 µL

Δ **Note:** For detecting the reduced form of glutathione only, omit Glutathione Reductase. Use 20 µL of the Glutathione Reaction Buffer to replace the 20 µL of Glutathione Reductase.

8.2 Mix well. Add 160 µL of the Reaction Mix to each well and incubate at room temperature for 10 min to generate NADPH.

8.3 Add 20 µL of either the GSH Standard/GSH standard solutions or the sample solution. Incubate the plate at room temperature for 5-10 min.

Δ **Note:** We recommend to make several dilutions of your sample using the 1% SSA to make sure the readings are within the range of the standard calibration curve.

8.4 Add 20 µL of Substrate solution, and incubate at room temperature for 5-10 min, or longer if the samples contain low levels of glutathione.

Δ **Note**:

- Since the reaction starts immediately after the addition of substrate, use of a multichannel pipette or repeating pipette is recommended to avoid the reaction time lag among wells.
- You can read samples immediately and at various times following addition of the substrate solution for kinetic studies.

8.5 Read the absorbance at 405 nm or 415 nm using a microplate reader.

8.6 Determine concentrations of GSH in the sample solutions using the standard glutathione calibration curve.

Δ **Note:**

- Using reduced form glutathione Standard Curve for detecting reduced form of glutathione. Using total Glutathione Standard Curve for detecting total glutathione.

- There are about 10 to 100 fold difference in detection sensitivity between detecting reduced form glutathione and total glutathione.
- The colorimetric reaction is stable and the O.D. increases linearly over 30 min for total glutathione detection.

9. Data Analysis

9.1 Calculation of reduced Glutathione:

9.1.1 Calculation of reduced glutathione in well (B µg)

$$Reduced\ Glutathione\ (B) = \frac{(OD_{sample} - OD_{blank})}{Slope_{STD\ Curve}} = \mu g$$

9.1.2 Calculation of reduced glutathione concentration in sample

$\frac{B}{V} = \mu g/ml\ sample$

Where:
B is the µg of reduced glutathione in the well
V is the volume of sample added to the well (0.02 ml)

9.2 Calculation of Total Glutathione (H ng):

9.2.1 Pseudo-end point method:

$$Total\ Glutathione = \frac{(OD_{sample} - OD_{blank})}{Slope\ STD\ Curve} = ng$$

9.2.2 Kinetic method (strongly recommended):

$$Total\ Glutathione = \frac{(Slope_{sample} - Slope_{blank})}{Slope_{STD\ Curve}} = ng$$

NOTE: Running this assay in kinetic mode shows kinetic curves for each of the standards. The kinetic curve of samples should fit within these curves at all times points to be within the linear range of the assay. If the sample curve intersects standard kinetic curves, this is indicative of slowed reaction within the sample, likely caused by not diluting the sample sufficiently.

9.2.3 Calculation of total glutathione concentration in sample

$$\frac{H}{V * 1000} * D = \mu g/ml\ sample$$

Where:
H is the ng of total glutathione in the well
V is the volume of sample added to the well (0.02 ml)
D is the dilution factor applied to the sample for it to fall within the standard curve for total glutathione (typically ~100).

9.3 Alternate Calculations

9.3.1 Standard curve setup.
Without changing any part of the protocol, the standard curves may be plotted in terms of nmol and pmols, instead of µg and ng, respectively, using the table below.

	Reduced Glutathione (in well)		Total Glutathione (in well)	
Standard #	GSH standard (µg)	Molar Equivalent (nmoles)	GSH standard (ng)	Molar Equivalent (pmoles)
1	10	32.5	100	325
2	8	26	80	260
3	6	19.5	60	195
4	4	13	40	130
5	2	6.5	20	65
6	0	0	0	0

9.3.2 Calculation of reduced glutathione in well (J, nmol)
All calculations remain the same, but using the standard curve where the X-axis is in nmoles will give an output of nmoles of reduced glutathione.

$$Reduced\ Glutathione\ (J) = \frac{(OD_{sample} - OD_{blank})}{Slope_{STD\ Curve}} = nmol$$

$$\frac{J}{V} = nmol/ml\ sample = \mu M$$

Where:
J is the nmol of reduced glutathione in the well
V is the volume of sample added to the well (0.02 ml)

9.3.3 Calculation of Total Glutathione
Kinetic method (strongly recommended)pmol (K) in well:

$$Total\ Glutathione\ (K) = \frac{(Slope_{sample} - Slope_{blank})}{Slope_{STD\ Curve}} = pmol$$

Calculation of total glutathione concentration in sample

$$\frac{K}{V * 1000} * D = nmol/ml\ sample = \mu M$$

Where:
K is the pmol of total glutathione in the well
V is the volume of sample added to the well (0.02 ml)
D is the dilution factor applied to the sample for it to fall within the standard curve for total glutathione (typically ~100).

9.3.4 Relating Total and reduced glutathione
NOTE: Caution must be taken as this will not be applicable for many samples (physiological GSSG concentrations are typically >100-fold lower than GSH). If the total glutathione is less than reduced glutatione, the amount of GSSG is below the limit of detection and the difference may be in part due to the sensitivity of the reduced glutathione assay. Significant figures should be considered.

$$\frac{Total\ Glutathione - \ Reduced\ Glutatione(GSH)}{2} = GSSG\ (\mu M)$$

10. Typical Data

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

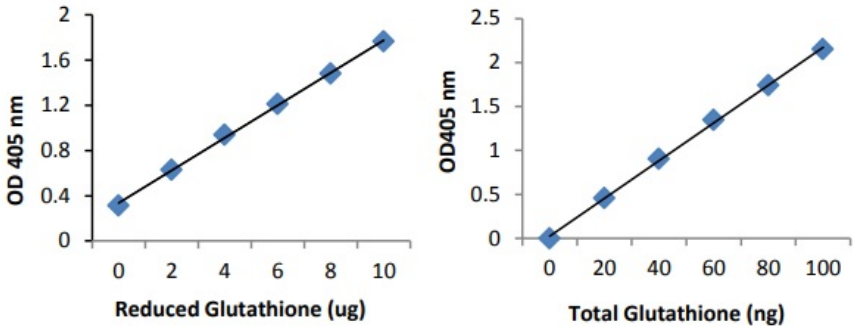

Figure 1. Glutathione Standard Curve. Various amounts of standard glutathione was added to the glutathione reaction and incubated for 10 min according to the kit instructions. Absorbance was measured at O.D. 405 nm.

11. Reagent Interference

Reducing agents such as ascorbic acid, β-mercaptoethanol, dithiothreitol (DTT) and cysteine, or thiol reactive compounds such as maleimide compounds, interfere with the glutathione assay and therefore should be avoided during the sample preparation.

When detecting the reduced form of glutathione, protein thiols can generate significant background signal. In such cases, it is necessary to completely remove proteins from samples. We suggest using ab93349 - 10kD Spin Column to remove proteins. Then the reduced glutathione can be easily detected from spin through samples.

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)