

May 15, 2019

UC Davis - Glutathione

DOI

dx.doi.org/10.17504/protocols.io.ykcfusw



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DOI: <https://dx.doi.org/10.17504/protocols.io.ykcfusw>

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Protocol Citation: Peter Havel 2019. UC Davis - Glutathione. protocols.io <https://dx.doi.org/10.17504/protocols.io.ykcfusw>

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Protocol status: Working

We use this protocol and it's working

Created: February 26, 2019

Last Modified: May 15, 2019

Protocol Integer ID: 20836

Keywords: Glutathione, using glutathione reductase, use of glutathione reductase, glutathione reductase for the quantification, glutathione reductase, total glutathione, glutathione summary, glutathione, sulfhydryl group of gsh react, gsh in the sample, gsh react, gsh measurement, disulfide dimer gssg, accurate estimation of gsh, enzymatic recycling method, optimized enzymatic recycling method, gsh, mixed disulfide, assay, cayman gsh, nitrobenzoic acid, proportional to this recycling reaction, acid, recycling reaction, deproteination before assay, rate of tn timer production, gstn timer, tn timer production

Abstract

Summary:

Cayman's GSH assay kit utilizes a carefully optimized enzymatic recycling method, using glutathione reductase for the quantification of GSH. The sulfhydryl group of GSH reacts with DTNB (5,5'-dithio-bis-2-nitrobenzoic acid, Ellman's reagent) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB) that is concomitantly produced, is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to this recycling reaction which in turn is directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of TNB at 405 or 412 nm provides an accurate estimation of GSH in the sample. GSH is easily oxidized to the disulfide dimer GSSG. Because of the use of glutathione reductase in the Cayman GSH assay kit, both GSH and GSSG are measured and the assay reflects total glutathione. The kit can also be used to measure only GSSG by following an alternative protocol. GSH measurement can be done in plasma, tissue samples, and cultured cells using this kit. Nearly all samples require deproteination before assay.



Materials

MATERIALS

⊗ Assay Kit **Cayman Chemical Company Catalog #703002**

⊗ Buffer

⊗ Standard

⊗ Co-Factor Mix

⊗ Enzyme Mix

⊗ DTNB

Note:

Cayman Chemical RRID:SCR_008945

Troubleshooting

- 1 Add 50 μ l of Standard (tubes A-H) per well in the designated wells on the plate (see **Sample Plate Format**, Figure 2, page 13).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	B	B	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	C	C	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	D	D	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	E	E	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	F	F	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	G	G	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	H	H	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

A-H = Standards

S1-S40 = Sample Wells

Figure 2. Sample plate format

- 2 Add 50 μ l of sample to each of the sample wells.
- 3 Cover the plate with the plate cover provided.
- 4 Prepare the Assay Cocktail by mixing the following reagents in a 20 ml vial: MES Buffer (11.25 ml), reconstituted Cofactor Mixture (0.45 ml), reconstituted Enzyme Mixture (2.1 ml), water (2.3 ml), and reconstituted DTNB (0.45 ml). **NOTE:** *The volumes of reagents given are for the use of the entire plate. Adjust the volumes of the reagents accordingly if only a part of the plate is used. Prepare fresh Assay Cocktail and run a standard curve each time the assay is performed. Use the Assay Cocktail within 10 minutes of preparation.*
- 5 Remove the plate cover and add 150 μ l of the freshly prepared Assay Cocktail to each of the wells containing standards and samples using a multichannel pipette. Replace the plate cover and incubate the plate in the dark on an orbital shaker.

- 6 Measure the absorbance in the wells at 405–414 nm using a plate reader at five minutes intervals for 30 minutes (a total of 6 measurements). **NOTE:** *If only the end point method of calculation (see page 18) is used, one measurement at 25 minutes is enough. Expected absorbance of the lowest standard (standard A) at 405 nm is 0.15–0.25 AU and that of the highest standard (standard H) is 0.6–0.8 AU in 30 minutes.*

7 Calculations

GSH concentration of the samples can be determined either by the End Point Method or the Kinetic Method. The End Point Method is adequate for most purposes. However, if the levels of cysteine or other thiols in the samples are expected to be significant compared to GSH, the Kinetic Method should be used.

End Point Method

1. Calculate the average absorbance from the 25 minutes measurement for each standard and sample.
2. Subtract the absorbance value of the standard A from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Plot the corrected absorbance values (from step 2 above) of each standard as a function of the concentration of GSSG or Total GSH of Table 1 (see Figure 3, on page 18).

Tube	GSSG Standard (μl)	MES Buffer (μl)	Final Concentration (μM GSSG)	Equivalent Total GSH (μM)*
A	0	500	0	0
B	5	495	0.25	0.5
C	10	490	0.5	1.0
D	20	480	1.0	2.0
E	40	460	2.0	4.0
F	80	420	4.0	8.0
G	120	380	6.0	12.0
H	160	340	8.0	16.0

Table 1. Glutathione standards

*under the assay conditions GSSG is reduced to produce 2 mole equivalents of GSH.

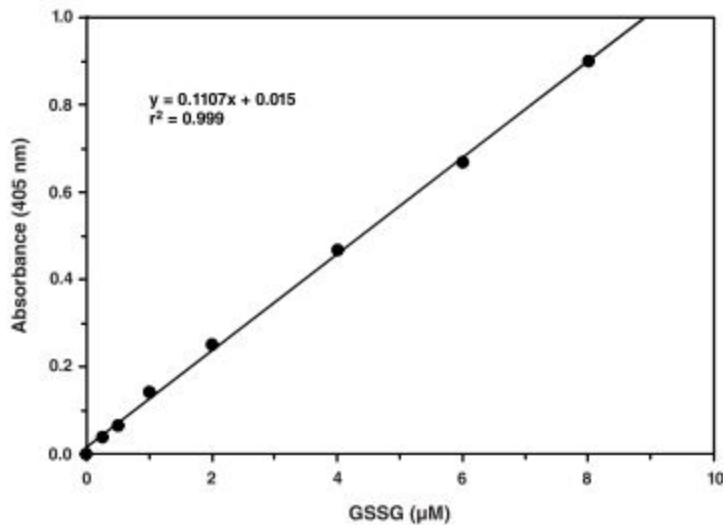


Figure 3. Plot of corrected absorbance at 25 minutes *versus* GSSG concentration (μM)

4. Calculate the values of GSSG or Total GSH for each sample from the standard curve.

[Total GSH] or [GSSG] =

$$\left[\frac{(\text{Absorbance at 405-414 nm}) - (\text{Y-intercept})}{\text{Slope}} \right] \times 2^* \times \text{Sample Dilution}$$

NOTE: If your sample required deproteinization, multiply by "2" to account for the addition of MPA Reagent.