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UC Davis - Glutathione

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Protocol status: Working
We use this protocol and it's working

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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 deproteinization before assay.

Keywords: Glutathione

MATERIALS

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- Assay Kit Cayman Chemical Company Catalog #703002
- Buffer Contributed by users
- Standard Contributed by users
- Co-Factor Mix Contributed by users
- Enzyme Mix Contributed by users
- DTNB Contributed by users

Note:

Cayman Chemical [RRID:SCR_008945](#)

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

A-H = Standards
S1-S40 = Sample Wells

Figure 2. Sample plate format

- 2 Add 50 µl 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 µl 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.