Glutathione Protocol

Version: 1
Replaced by version
Edited by: Peter Havel - UC Davis Metabolism and Endocrinology Core

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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Assay Kit	Cayman	703002
Buffer		
Standard		
Co-Factor Mix		
Enzyme Mix		
DTNB		

Protocol:

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).
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.*

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).