Thiolite™ Green

Ordering Information	*Storage Conditions*
Product Number: 21508 (5 mg)	Store at -20 °C, desiccated and protected from light Expiration date is 12 months from the date of receipt

Introduction

Thiolite™ Green is one of the most sensitive sensors for measuring thiol compounds. It gives a green fluorescent adduct upon reacting with thiol compounds (such as cysteine). It can be used to quantify the number of cysteines on a protein. When we use it to measure glutathione fluorimetrically, it has >200 fold fluorescence enhancement upon reaction with thiol-containing compounds.

Chemical and Physical Properties

Molecular Weight: ~400
Solvent: dimethylsulfoxide (DMSO)
Spectral Properties: Excitation = 510 nm; Emission = 524 nm

Assay Protocol with Thiolite™ Green in a 96-well Plate

Brief Summary

Prepare Thiolite™ Green working solution (50 µL) → Add GSH standards or test samples (50 µL) → Incubate at room temperature for 10 min-1 hr → Read fluorescence intensity at Ex/Em = 490/525 nm

Note: Following is our recommended protocol for thiol assay in solution. This protocol only provides a guideline, and should be modified according to your specific needs.

1. Prepare Thiolite Green™ working solution:

1.1 Prepare a 10 to 25 mM stock solution of Thiolite™ Green in high-quality, anhydrous DMSO. The stock solution should be used promptly; any remaining solution need be aliquoted and frozen at -20 °C.
Note: The unused Thiolite Green stock solution should be divided into single use aliquots and stored at -20°C, protected from light.

1.2 Prepare a 2X Thiolite™ Green working solution: On the day of the experiment, either dissolve Thiolite™ Green in DMSO or thaw an aliquot of the Thiolite™ Green stock solution to room temperature. Prepare a 2X working solution at the final concentration ranging from 100 to 250 µM in 20 mM Hepes buffer or buffer of your choice, pH 7. It is recommended to use Thiolite™ Green at the final concentration ranging from 50 to 100 µM to measure Thiol concentration in solution.

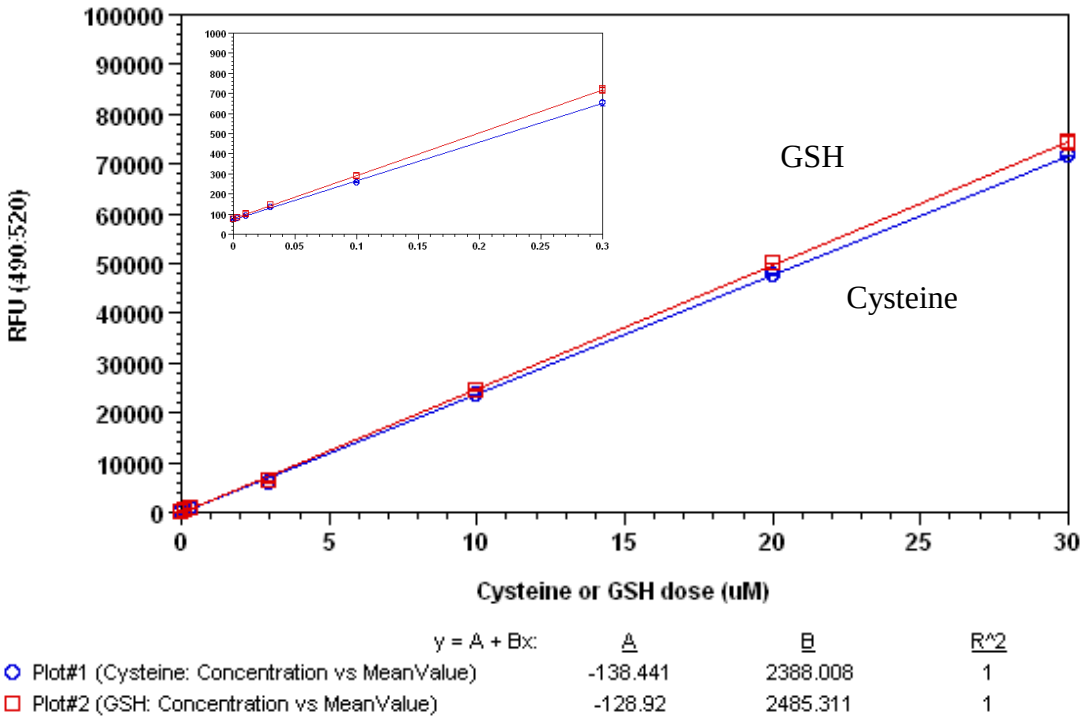
2. Run GSH Assay in supernatants:

2.1 Add 50 µL of 2X Thiolite™ Green working solution (from Step 1.2) to each well of the GSH standard, blank control, and test samples to make the total GSH assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of GSH reaction mixture into each well.

2.2 Incubate the reaction at room temperature for 10 to 60 minutes, protected from light.

2.3 Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 490/525 nm.

2.4 The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the thiol reaction.



	y = A + Bx:	A	B	R^2
Plot#1 (Cysteine: Concentration vs MeanValue)		-138.441	2388.008	1
Plot#2 (GSH: Concentration vs MeanValue)		-128.92	2485.311	1

Figure 1. GSH and cysteine dose response was measured with Thiolite™ Green on a 96-well black plate using a NOVOStar microplate reader (BMG Labtech). As low as 10 nM (1 pmol/well) of GSH or cysteine can be detected with 10 minutes incubation time (n=3). The insert shows the low levels of thiol detection.

Disclaimer: *This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact our technical service representative for more information.*