Thiolite™ Blue

Ordering Information	*Storage Conditions*
Product Number: 21507 (5 mg)	Store at -20 °C, desiccated and protected from light Expiration date is 12 months from the date of receipt

Introduction

Thiolite™ Blue is one of the most sensitive sensors for measuring thiol compounds. It gives a blue fluorescent adduct upon reacting with thiol compounds (such as GSH and cysteine). It can be used to quantifying the number of cysteines on a protein. We have used it to measure glutathione fluorimetrically. It has >200-fold fluorescence enhancement upon reaction with thiol-containing compounds. Thiolite™ Blue is an excellent replacement for mBBr (monobromobimane) due to their similar spcetral properties. Compared to mBBr, the thiol adduct of Thiolite™ Blue has much stonger fluorescence and absorption than that of mBBr, making it a much more sensitive thiol probe than bromobimanes.

Chemical and Physical Properties

Molecular Weight: 289.24
Solvent: dimethylsulfoxide (DMSO)
Spectral Properties: Excitation = 335 nm; Emission = 460 nm

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

Brief Summary

Prepare Thiolite™ Blue 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 = 335/460 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 Blue™ working solution:

1.1 Prepare a 10 to 25 mM stock solution of Thiolite™ Blue 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 Blue stock solution should be divided into single use aliquots and stored at -20 °C, protected from light.

1.2 Prepare a 2X Thiolite™ Blue working solution: On the day of the experiment, either dissolve Thiolite™ Blue in DMSO or thaw an aliquot of the Thiolite™ Blue 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™ Blue 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™ Blue 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 = 335/460 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.

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