# Thiolite™ Blue, AM

| *Ordering Information* | *Storage Conditions* |
|---|---|
| Product Number: 21506 (1 mg) | Store at -20 °C, desiccated and protected from light<br>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. Thiolite™ Blue is optimized for intracellular thiol detection. It is non-fluorescent outside cells, eliminating the wash step and reducing assay background.

## Chemical and Physical Properties

Molecular Weight: 331.28
Solvent: dimethylsulfoxide (DMSO)
Spectral Properties: Excitation = 335 nm; Emission = 460 nm

## Example of Assay Protocol with Thiolite™ Blue AM for Flow Cytometer

### Brief Summary

**Prepare cells with test compounds → Add 1X dye working solution → Incubate dyes with cells at room temperature or 37 °C for 10 to 30 min → Remove the dye working solution → Analyze with a flow cytometer at ex/Em = 335/460 nm**

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

**1. Prepare Thiolite Blue™ AM working solution:**

Prepare a 1 to 5 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.*

**2. Prepare 1X dye working solution**

Prepare a 1X dye working solution by diluting the DMSO stock solution (from Step 1) in Hanks and 20 mM Hepes buffer (HHBS) or the buffer of your choice, pH 7 right before use. Mix them well by vortexing.
*Note: The final concentration of the dye working solution should be empirically determined for different cell types and/or experimental conditions. It is recommended to test at the concentrations that are at least over a ten fold range. The recommend concentration in Jurkat cells is 1-10 uM.*

**3. Analyze cells with a flow cytometer or a fluorescence microscope:**

3.1 Treat cells with test compounds for a desired period of time.

3.2 Centrifuge the cells to get 1-5 × $10^5$ cells per tube.

3.3 Resuspend cells in 1 mL of the dye working solution (from Step 2).
*Optional: One can add the DMSO stock solution into the cells directly without medium removing (such as, add 1 µLof 1 mM DMSO stock solution into 1 mL cells)*

3.4 Incubate cells with a dye solution at room temperature or 37 °C for 10 to 30 min, protected from light.

3.5 Remove the dye working solution from the cells, wash the cells with HHBS or buffer of your choice. Resuspend cells in 1 mL of pre-warmed HHBS or medium to get 2-10 × $10^5$ cells per tube.

3.6 Monitor the fluorescence change at Ex/Em 335/460 nm with a flow cytometer or a fluorescence microscope.