Thiolite™ Blue, AM

Catalog number: 21506
Unit size: 1 mg

Component	Storage	Amount
Thiolite™ Blue, AM	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

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 spectral 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.

KEY PARAMETERS

Flow cytometer

Excitation	350 nm or 405 nm laser
Emission	450/40 nm filter
Instrument specification(s)	Pacific Blue channel

Fluorescence microscope

Excitation	DAPI filter set
Emission	DAPI filter set
Recommended plate	Black wall/clear bottom

Fluorescence microplate reader

Excitation	340
Emission	460
Cutoff	420
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Thiolite™ Blue AM Stock Solution
Prepare a 1 to 5 mM stock solution of Thiolite™ Blue in high-quality, anhydrous DMSO.

PREPARATION OF WORKING SOLUTION

Thiolite™ Blue AM Working Solution
On the day of the experiment, either dissolve Thiolite™ Blue AM in DMSO or thaw an aliquot of the indicator stock solution to room temperature. Prepare a 1X Thiolite™ Blue AM working solution in a buffer of your choice (e.g., Hanks and Hepes buffer, pH 7), and 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 concentrations that are at least over a ten-fold range. The recommended concentration in Jurkat cells is 1-10 µM.

SAMPLE EXPERIMENTAL PROTOCOL

The following is a recommended protocol for loading Thiolite™ Blue AM into live mammalian cells. This protocol only provides a guideline, should be modified according to your specific needs.

1. Prepare viable cells as desired.
2. Treat cells with test compounds.
3. Centrifuge the cells to get 1-5 × 10^5 cells per tube.
4. Resuspend cells in 1 mL of Thiolite™ Blue AM working solution.

   Note Alternatively, the DMSO stock solution can be added directly to the cells without removing the medium. For example, add 1 µL of 1 mM DMSO stock solution into 1 mL cells
5. Incubate the dye-loaded plate in a cell incubator at 37 °C for 15 to 60 minutes.
6. Remove the dye working solution and wash cells with HHBS or buffer of your choice to remove any excess probes.
7. Resuspend the cells in 1 mL of pre-warmed HHBS or medium to get 2-10 × 10^5 cells per tube.
8. Measure the fluorescence intensity using either a fluorescence microscope equipped with a DAPI filter set, a flow cytometer equipped with a UV/violet laser and a 450/40 nm filter (Pacific Blue channel), or a fluorescence plate reader at Ex/Em = 340/460 nm cutoff 420 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

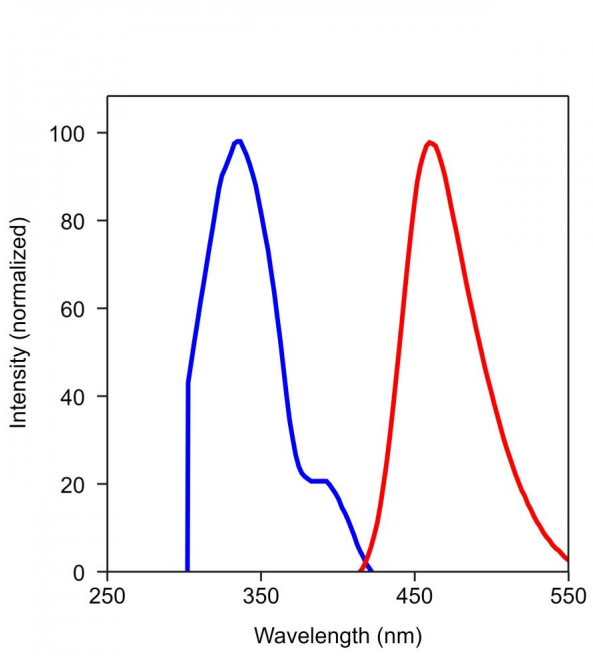

Figure 1.
The excitation and emission spectra of Thiolite™ Blue, AM.

DISCLAIMER

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