

Thiolite™ Blue, AM

Catalog number: 21506
Unit size: 1 mg

Component	Storage	Amount (Cat No. 21506)
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 stronger 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

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

Fluorescence microscope

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

Fluorescence microplate reader

Cutoff	420
Emission	460
Excitation	340
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

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 μ M.

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 $1-5 \times 10^5$ 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 μ L 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 $2-10 \times 10^5$ 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

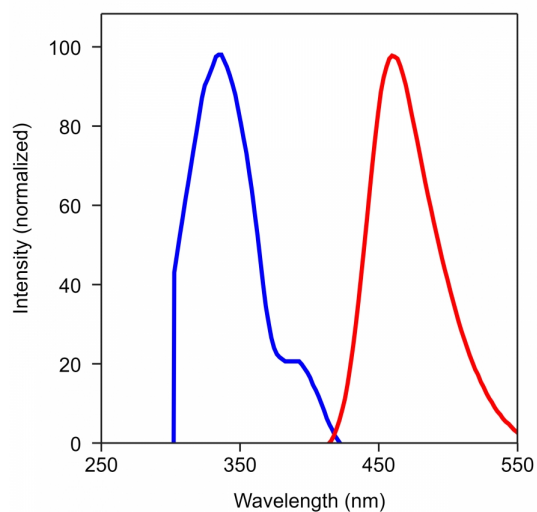


Figure 1. The excitation and emission spectra of Thiolite™ Blue, AM.

DISCLAIMER

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