ThiolTrace™ Violet 500

Catalog number: 22280
Unit size: 500 Tests

Component	Storage	Amount
ThiolTrace™ Violet 500	Freeze (<-15 °C), Minimize light exposure	500 tests

OVERVIEW

The subcellular detection and localization of GSH is important in understanding the modulation of redox status, the effect of drugs, and the mechanisms of detoxification. ThiolTrace™ Violet 500 is a brighter and more robust intracellular thiol probe for monitoring intracelluar GSH than the commonly used mBBr, mBCl or Thiotracker™ Violet. Since reduced glutathione represents the majority of intracellular free thiols in the cell, ThiolTrace™ Violet 500 can be used in estimating the cellular level of reduced glutathione. It is at least 10X brighter than mBCL and other intracellular common thiol detection probes (e.g., Thiotracker Violet), and can be excited with UV or 405 nm excitation with a large Stokes shift. It can be fixed with aldehydes and permeabilized by Triton® X-100 (0.5%). It may be used in multiplex assays including cytotoxicity studies. ThiolTrace Violet 500 provides a simple, sensitive and reproducible tool to detect reduced GSH content in biological samples. ThiolTrace Violet 500 reacts with thiol to emit a strong fluorescence of 520-530 nm with excitation at the common violet 405 nm laser. When compared with ThiolTracker Violet (Thermo Fisher Scientific), ThiolTrace Violet 500 has 10-100 fold higher intensity in cell culture medium containing growth factors. ThiolTrace Violet 500 is compatible with wide variety of diluents including serum containing cell culture medium. ThiolTrace™ Violet 500 can be used for Flow Cytometry, HCS imaging and epifluorescent microscopy.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds at a density of 5×10^5 to 1×10^6 cells/mL
2. Prepare and add ThiolTrace™ Violet 500 working solution to cells
3. Incubate at 37°C for 20 to 30 minutes
4. Read fluorescence intensity at Ex/Em = 405/525 nm-Pacific Orange filter set

Important Thaw at room temperature before starting the experiment.

Note **For flow cytometry and fluorescence microscopy, 200 and 500 tests can be performed with the quantity provided, respectively.**

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	405 nm laser
Emission:	525/50 nm filter
Instrument specification(s):	Pacific Orange channel

Instrument:	Fluorescence microscope
Excitation:	405 nm
Emission:	525 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	TRITC filterset

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.

ThiolTrace™ Violet 500 stock solution (500X):
Add 200 µL of DMSO (Not provided) into the vial of ThiolTrace™ Violet 500, and mix well.

Note Aliquot and stored the unused ThiolTrace™ Violet 500 stock solution at -20°C. Avoid repeated freeze/thaw cycles.

PREPARATION OF WORKING SOLUTION

ThiolTrace™ Violet 500 working solution (1X):
Add 1 µL of ThiolTrace™ Violet 500 stock solution into 0.5 mL of buffer of your choice, and mix well.

Note *ThiolTrace™ Violet 500 working solution can be prepared in the cell culture medium containing serum.*

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

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

   Note For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to the incubation with ThiolTrace™ Violet working solution.

   Note The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

2. Centrifuge the cells at 1000 rpm for 4 minutes, and wash cells in 1 mL of buffer of your choice (Optional).

3. Resuspend cells in 0.5 mL ThiolTrace™ Violet 500 working solution and incubate them at 37°C incubator for 20 to 30 minutes.

   Note For the fluorescence microscopy, add 200 µL of the ThiolTrace™ Violet 500 working solution per well.

4. Centrifuge the cells at 1000 rpm for 4 minutes, and then wash cells in 1 mL of buffer of your choice (Optional).

5. Resuspend in buffer and monitor the fluorescence intensity with a flow cytometer using Pacific Orange filter set (Ex/Em = 405/525 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

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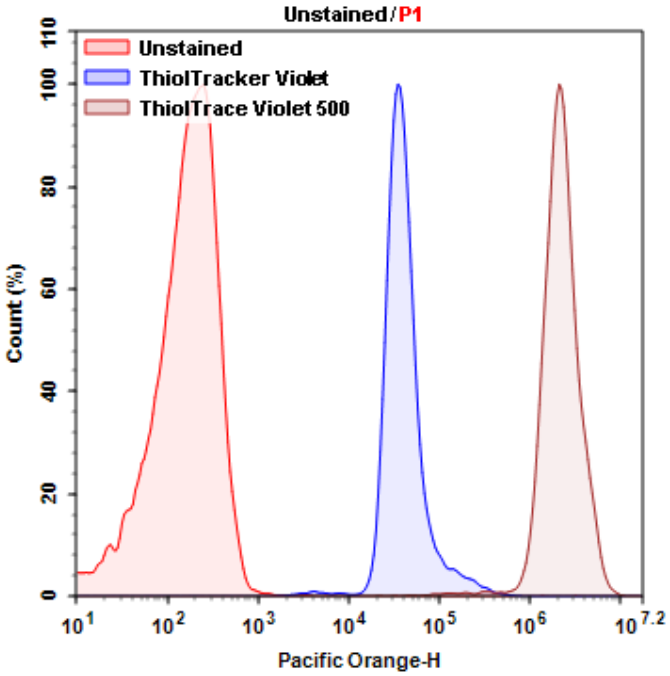

Figure 1. The comparison in the fluorescence intensity of ThiolTrace™ Violet 500 with ThiolTracker™ Violet (Thermo Scientific) in Jurkat cells in the **presence of cell culture medium**. Jurkat cells were dye loaded with ThiolTrace™ Violet 500 or ThiolTracker™ Violet for 20 minutes in a 37 °C, 5% CO_2 incubator. The fluorescence intensity was measured using ACEA NovoCyte 3000 flow cytometer with Pacific Orange channel.