ThiolTracker™ Violet (Glutathione Detection Reagent)

Catalog nos. T10095, T10096

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage*	Stability
ThiolTracker™ Violet	180 assays (T10095) 5 microplates (T10096)	Solid	• ≤–20˚C • Desiccate • Protect from light	When stored as directed the product is stable for 1 year
Number of assays: Sufficient material is supplied for T10095 for staining suspension cells in 180 tubes (0.5 mL/tube) for flow cytometric analysis. Sufficient material is supplied for T10096 for staining cells in 5 × 96-well microplates for imaging, or cells on approximately 120 of 5 mm round coverslips for imaging.				
Approximate fluorescence excitation/emission maxima: 404/526 in nm				

Introduction

ThiolTracker™ Violet dye is ideal for cellular labeling due to the following properties of the dye: (1) reacts actively with reduced thiols in intact cells, (2) can be efficiently excited with 405 nm laser or traditional xenon or mercury arc lamps for use in cellular analysis using flow cytometry and fluorescence microscopy, (3) excitation and emission property is well suitable for imaging with fluorescence microscopes equipped with filter set for Hoechst 33342 dye.

Glutathione (GSH) plays a central role in protecting mammalian cells against damage incurred by free radicals, oxidants, and electrophiles. Since reduced glutathione represents the majority of intracellular free thiols in cells, ThiolTracker™ Violet dye can be used in estimating the cellular level of reduced glutathione.

ThiolTracker™ Violet dye is applied directly to live cells in thiol-free buffer to stain the cells. After labeling, live cells can be directly imaged or cells can be fixed with aldehyde before imaging. Preservation of ThiolTracker™ Violet dye signal upon Triton® X-100 permeabilization step is excellent allowing ThiolTracker™ Violet dye labeled cells to be probed with antibodies or other probes.

Before You Begin

Materials Required but Not Provided

- Dimethyl sulfoxide (DMSO), high quality DMSO (>99.7 %, such as Sigma D-2650, anhydrous) is recommended.

- Dulbecco's phosphate buffered saline, containing Ca^{++} and Mg^{++}, glucose, and sodium pyruvate. (D-PBS with C/M, Invitrogen Cat. no. 14287-080)
- Dulbecco's phosphate buffered saline, without Ca^{++} or Mg^{++}, (D-PBS, Invitrogen Cat. no. 14190-144)
- Formaldehyde 37% aqueous solution

Preparing Cells

Seed cells the day before addition of the test compound. For adherent cells, optimize the cell number and plate coating requirements for the chosen cell model and time span of test compound treatment before labeling with ThiolTracker™ Violet dye. While many cell lines tested do not require special coating of surface, it is highly recommended to seed HepG2 cells on poly-lysine coated surface for staining with ThiolTracker™ Violet dye. Similar coating requirements might be applicable to other cell lines. Detachment of cells upon labeling with ThiolTracker™ Violet dye may indicate the need for optimization of dye concentration, labeling time, and substratum requirements.

ThiolTracker™ Violet

Follow these guidelines for preparing and storing ThiolTracker™ Violet dye:

- Prepare the ThiolTracker™ Violet dye stock solution in DMSO as described in the protocol. Alternatively, you can use 1-methyl-2-pyrrolodine to prepare the dye.
- It may take up to ten minutes to completely dissolve ThiolTracker™ Violet dye in DMSO. Pipeting up and down facilitates dye solubilization into DMSO and appears to work better than vortexing.
- In the protocols below, the ThiolTracker™ Violet dye stock solution concentration in DMSO is 20 mM. The working concentration is 20 µM for imaging and 10 µM for flow cytometry. Lower concentrations can be chosen for the stock solution to suit the optimized working concentrations of ThiolTracker™ Violet dye for a cell line of interest.
- Once reconstituted in DMSO, use the reconstituted ThiolTracker™ Violet dye **immediately**. Store any remaining unused stock solution of ThiolTracker™ Violet dye at –20˚C and use the stock solution within 2 weeks of preparation. Avoid repeated freeze-thaw steps.

Labeling Guidelines

Since ThiolTracker™ Violet is a thiol reactive dye, use **thiol-free buffer** for labeling live cells. Our studies indicate that labeling cells with 20 µM ThiolTracker™ Violet dye for 30 minutes at 37˚C gives excellent results in reporting the increase or decrease of stain intensity after treatment of cells with agents that modulate the intracellular reduced glutathione levels. However, some optimization of the working dye concentration and labeling time for your cell line of interest may be necessary. Many cell lines tested were found to be labeled well with ThiolTracker™ Violet dye ranging from 10 µM to 20 µM with the protocols outlined below.

Experimental Protocol for Labeling Cells in 96-well Plates for Imaging

Preparing Dye Stock Solution

Prepare ThiolTracker™ Violet dye in DMSO as follows to obtain a final concentration of 20 mM:

- For Cat. no. T10095, add 15 µL DMSO to each vial
- For Cat. no. T10096, add 75 µL DMSO to each vial

Preparing Dye Working Solution

Prepare ThiolTracker™ Violet dye working solution **just before use** by diluting the ThiolTracker™ Violet dye stock solution into D-PBS with C/M to the working concentration.

For example, you need 100 µL of working dye solution for each well of a 96-well plate. Prepare 12 mL of **20 µM** ThiolTracker™ Violet dye working solution by diluting 12 µL of 20 mM ThiolTracker™ Violet stock solution into 12 mL of D-PBS with C/M for one 96-well plate.

Protocol for Labeling Cells in 96-well Plates

1.1 After test compound or drug treatment of cells (if required), remove the incubation medium from the wells of the 96-well plate.

1.2 Rinse cells twice with 100 µL D-PBS C/M per well. Remove the D-PBS C/M.

1.3 Add 100 µL prewarmed ThiolTracker™ Violet dye working solution in D-PBS C/M. Incubate the plate in a 37˚C cell culture incubator for 30 minutes.

1.4 Replace the ThiolTracker™ Violet dye working solution with a suitable buffer or medium.

1.5 The cells are now ready for imaging using a fluorescence microscope or other suitable fluorescence imaging instrument. The approximate fluorescence excitation and emission wavelength for ThiolTracker™ Violet dye is 404/526 in nm.

1.6 **Optional:** Perform optional formaldehyde fixation as follows:

a. Prepare 3–4% formaldehyde solution in D-PBS. You need 100 µL/well for a 96-well plate.

b. Remove the ThiolTracker™ Violet dye working solution and add 100 µL 3–4% formaldehyde in D-PBS to each well.

c. Incubate the plate in a fume hood at room temperature for 30 minutes.

d. Remove the formaldehyde fixative and rinse cells twice with D-PBS. The cells are now ready for imaging.

Note: We recommend imaging the cells immediately after labeling and processing. If the plate cannot be imaged immediately, keep the processed plate at 4˚C.

1.7 **Optional:** Perform optional counterstaining for fixed samples. For example, counterstain for nucleus using nucleic acid dyes for cell identification purposes often employed in automated image analysis (high-content analysis).

Note: We recommend imaging the cells immediately after labeling and processing. If the plate cannot be imaged immediately, keep the processed plate at 4˚C.

Experimental Protocol for Labeling Cells on Coverslips for imaging

Preparing Dye Stock Solution

Prepare ThiolTracker™ Violet dye in DMSO as follows to obtain a final concentration of 20 mM:

- For Cat. no. T10095, add 15 µL DMSO to each vial
- For Cat. no. T10096, add 75 µL DMSO to each vial

Preparing Dye Working Solution

Prepare ThiolTracker™ Violet dye working solution **just before use** by diluting the ThiolTracker™ Violet dye stock solution into D-PBS with C/M to the working concentration.

Prepare enough dye working solution for the type of cell culture dish holding the coverslips. For example, 1 mL of ThiolTracker™ Violet dye working solution in D-PBS with C/M is sufficient for coverslips in a well of a 6-well plate or a 3-cm cell culture dish. Required volume can be adjusted according to the size of coverslips and the type of cell culture vessel used.

Protocol for Labeling Cells on Coverslips

2.1 After test compound or drug treatment of cells (if required), remove the incubation medium.

2.2 Rinse cells twice with D-PBS C/M. Remove the D-PBS C/M.

2.3 Add the appropriate volume of prewarmed ThiolTracker™ Violet dye working solution in D-PBS C/M depending on the cell culture dish. Incubate cells in a 37°C cell culture incubator for 30 minutes.

2.4 Replace the ThiolTracker™ Violet dye working solution with a suitable buffer or medium.

2.5 The cells are now ready for imaging using a fluorescence microscope or other suitable fluorescence imaging instrument. The approximate fluorescence excitation and emission wavelength for ThiolTracker™ Violet dye is 404/526 in nm.

2.6 **Optional:** Perform optional formaldehyde fixation as follows:

a. Prepare 3–4% formaldehyde solution in D-PBS.

b. Remove the ThiolTracker™ Violet dye working solution and add 3–4% formaldehyde in D-PBS to each coverslip.

c. Incubate cells in a fume hood at room temperature for 30 minutes.

d. Remove the formaldehyde fixative and rinse cells twice with D-PBS. The cells are now ready for imaging.

Note: We recommend imaging the cells immediately after labeling and processing. If the plate cannot be imaged immediately, keep the processed plate at 4°C.

2.7 **Optional:** Perform optional counterstaining for fixed samples. For example, counterstain for nucleus using nucleic acid dyes for cell identification purposes often employed in automated image analysis (high-content analysis).

Note: We recommend imaging the cells immediately after labeling and processing. If the plate cannot be imaged immediately, keep the processed plate at 4°C.

Experimental Protocol for Labeling Suspension Cells for Flow Cytometry

Preparing Dye Stock Solution

Prepare ThiolTracker™ Violet dye in DMSO as follows to obtain a final concentration of 10 mM:

- For Cat. no. T10095, add 30 µL DMSO to each vial
- For Cat. no. T10096, add 150 µL DMSO to each vial

Preparing Dye Working Solution

Prepare ThiolTracker™ Violet dye working solution **just before use** by diluting the ThiolTracker™ Violet dye stock solution into D-PBS with C/M to 2X working concentration.

Use a working concentration of 10 µM as a starting concentration for optimization. Required volume of working dye solution is 250 µL per tube for a final volume of 500 µL

Protocol for Labeling Suspension Cells

3.1 After test compound or drug treatment of cells (if required), remove the incubation medium by centrifugation.

3.2 Add the appropriate volume of D-PBS with C/M to wash cells. Pellet cells by centrifugation and discard the supernatant..

3.3 Resuspend the cells in an appropriate volume of D-PBS with C/M. Count cells and then adjust the cell density to 2X the desired final cell density. Dispense 250 µL cell suspension in a sterile test tube.

3.4 Add 250 µL 2X ThiolTracker™ Violet dye working solution in D-PBS with C/M to each tube containing 250 µL of cells in suspension. Gently mix. Incubate cells in a 37°C cell incubator for 30 minutes, protected from light.

3.5 Wash cells once with D-PBS or other buffer. Pellet cells by centrifugation and discard the supernatant. Resuspend cells in D-PBS with C/M or other buffer. Acquire data using a flow cytometer at 405 nm excitation and 525BP or similar emission filter.

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat. no.	Product Name	Unit Size
T10095	ThiolTracker™ Violet (Glutathione Detection Reagent) *for 180 assays*	1 set of 3 vials
T10096	ThiolTracker™ Violet (Glutathione Detection Reagent) *for 5 microplates*	each

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