Cell Meter™ Intracellular GSH Assay Kit *Optimized for Flow Cytometry with 405 nm excitation*

Catalog number: 22809
Unit size: 100 Tests

Component	Storage	Amount
Component A: ThiolTrace™ Violet 500	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (100 mL)
Component C: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

There are a variety of parameters that can be used for monitoring cell apoptosis. This particular kit is designed to detect cell apoptosis by measuring the decrease in reduced glutathione (GSH). GSH is important for maintaining redox level of cells. It is involved in many cellular processes including the scavenging of free radicals, drug detoxification, cell signaling, and cell proliferation. The decrease in cellular GSH concentration is an early hallmark in the progression of cell death in response to different apoptotic stimuli in many cells. Our Cell Meter™ Intracellular GSH Assay Kit uses our proprietary ThiolTrace™ Violet 500, which becomes strongly fluorescent upon reacting with thiol (including GSH in cells). In normal cells, probe is highly fluorescent, while in apoptotic cells, staining intensity is decreased. Cells stained with ThiolTrace™ Violet can be visualized with a flow cytometer at Ex/Em = 405/525 nm (Pacific Orange filter set). The kit can be used together with other reagents, such as 7-AAD (#17501) for multi-parametric study of cell viability and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

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.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	405 nm
Emission:	525 nm
Instrument specification(s):	Pacific Orange 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 (Component C) 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 2 µL of ThiolTrace™ Violet 500 stock solution into 1 mL of Assay Buffer (Component B) or 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 1 mL ThiolTrace™ Violet 500 working solution and incubate them at 37°C incubator for 20 to 30 minutes.

4. 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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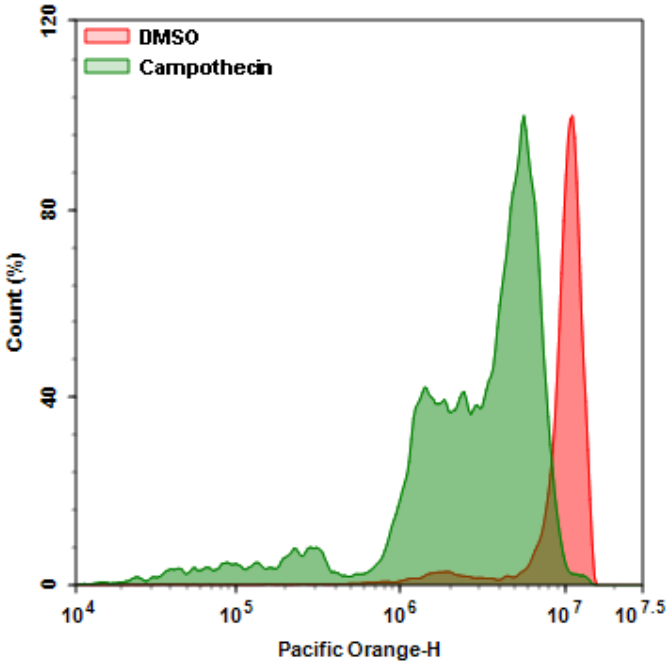

Figure 1. The decrease in the fluorescence intensity of ThiolTrace™ Violet 500 with the addition of Campothecin in HL-60 cells. HL-60 cells were treated for 6 hours without (Red line) or with 20 µM Campothecin (Green line) in a 37 °C, 5% CO_2 incubator, and then stained with ThiolTrace™ Violet 500 for 20 minutes. The fluorescence intensity was measured using ACEA NovoCyte 3000 flow cytometer with Pacific Orange channel.

DISCLAIMER