Cell Meter™ Intracellular GSH Assay Kit
Optimized for Flow Cytometry

Catalog number: 22810
Unit size: 100 Tests

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

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used for monitoring cell viability. This particular kit is designed to monitor cell apoptosis through measuring the depleted antioxidant reduced glutathione (GSH). GSH 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 cell types. Our Cell Meter™ Intracellular GSH Assay Kit provides all the essential components with an optimized assay method for the detection of apoptosis in cells with the decreased GSH. This fluorometric assay is based on the detection of the GSH in cells using our proprietary non-fluorescent Thiolite™ Green dye that becomes strongly fluorescent upon reacting with thiol. In normal cells, Thiolite™ Green accumulates primarily in cytosol, but it is partially translocated to mitochondria in apoptotic cells while Thiolite™ Green staining intensity decreases. Cells stained with Thiolite™ Green can be visualized by flow cytometry. The kit can be paired with other reagents, such as 7-AAD (Cat# 17501), propidium iodide (Cat# 17517) for multi-parametric study of cell viability and apoptosis. The kit is optimized for screening of apoptosis activators and inhibitors by flow cytometry.

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. Add 5 µL of 200X Thiolite™ Green into 1 mL of cell solution
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 15 to 30 minutes
4. Pellet the cells and resuspend the cells in 1 mL of growth medium
5. Analyze cells using flow cytometer with FL1 channel

Important Thaw all the kit components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC channel

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.

1. Thiolite™ Green stock solution (200X):
Add 500 µL of DMSO (Component C) into the vial of Thiolite™ Green (Component A) and mix well to make 200X Thiolite™ Green stock solution. Protect from light.

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. For each sample, prepare cells in 1 mL warm medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.

   Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Treat cells with test compounds for a desired period of time to induce apoptosis.

3. Add 5 µL of 200X Thiolite™ Green stock solution into the treated cells.

4. Incubate the cells in a 37°C, 5% CO_2 incubator for 15 to 30 minutes.

   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 Thiolite™ Green dye-loading solution. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

5. **Optional:** Centrifuge the cells at 1000 rpm for 4 minutes and then re-suspend cells in 1 mL of Assay Buffer (Component B) or buffer of your choice.

6. Monitor the fluorescence intensity using a flow cytometer with FL1 channel (Ex/Em = 490/525 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

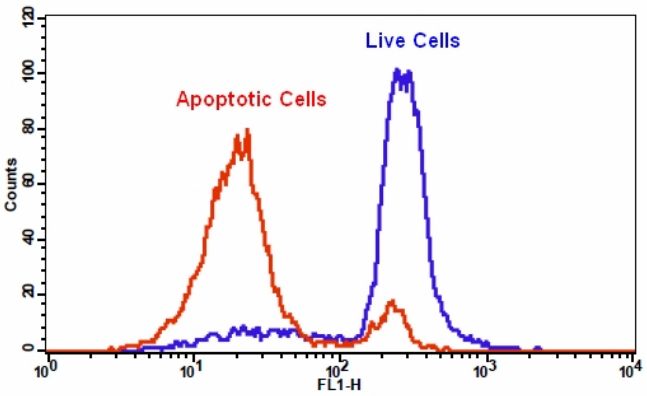

Figure 1. The decrease in the fluorescence intensity of Thiolite™ Green with the addition of camptothecin in Jurkat cells. Jurkat cells were treated overnight without (blue line) or with 20 µM camptothecin (red line) in a 37 °C, 5% CO2 incubator, and then dye loaded with Thiolite™ Green for 30 minutes. The fluorescence intensity of Thiolite™ Green was measured with a FACSCalibur (Becton Dickinson) flow cytometer using the FL1 channel.

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

DISCLAIMER

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