

Cell Meter™ Intracellular GSH Assay Kit

Optimized for Flow Cytometry

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22810 (100 assays)	Keep in freezer and avoid exposure to light	Flow Cytometer

Introduction

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 non-fluorescent Thiolite™ Green, which becomes strongly fluorescent upon reacting with thiol (including GSH in cells). In normal cells, Thiolite™ Green is accumulated primarily in cytosol, but it is partially translocated to mitochondria in apoptotic cells while Thiolite™ Green staining intensity is decreased. Cells stained with Thiolite™ Green can be visualized with a flow cytometer at Ex/Em = 490/520 nm (FL1 channel). 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.

Kit Components

Components	Amount
Component A: Thiolite™ Green	1 vial
Component B: Assay Buffer	1 bottle (100 mL)
Component C: DMSO	1 vial (500 µL)

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells with test compounds at a density of 5×10^5 to 1×10^6 cells/mL → Add 5 µL of 200X Thiolite™ Green into 1 mL of cell solution → Incubate at room temperature for 15-30 minutes → Pellet the cells and resuspend the cells in 1 mL of growth medium → Analyze with a flow cytometer using the FL1 channel

Note: Thaw all the kit components at room temperature before use.

1. Prepare cells:

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. Prepare 200X Thiolite™ Green:

Add 500 µL of DMSO (Component C) into the vial of Thiolite™ Green (Component A), and mix well.
Note: Aliquot and stored the unused Component A at -20°C. Avoid repeated freeze/thaw cycles.

3. Run GSH Green™ Assay:

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

3.2 Add 5 μ L of 200X Thiolite™ Green (from Step 2), and incubate the cells in a 37 °C, 5% CO₂ incubator for 15 to 30 minutes.

Note 1: 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.

Note 2: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

3.3 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.

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

Data Analysis

In live non-apoptotic cells, the green fluorescence intensity is increased when Thiolite™ Green is accumulated in cytosol and mitochondria. In apoptotic and dead cells, the fluorescence intensity of Thiolite™ Green is reduced by the decreased GSH.

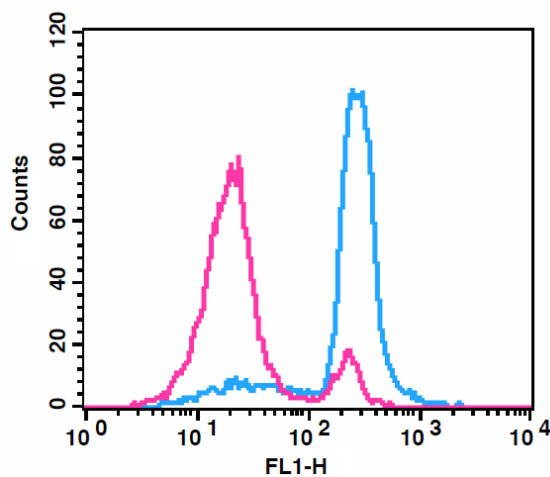


Figure 1. The decrease in the fluorescence intensity of Thiolite™ Green with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated overnight without (blue line) or with 20 μ M camptothecin (pink line) in a 37 °C, 5% CO₂ incubator, and then dye loaded with Thiolite™ Green for 30 minutes.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

Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.