

ab112132

Intracellular GSH Assay Kit

Instructions for Use

For detecting Intracellular GSH in cells by using our proprietary green fluorescence probe

This product is for research use only and is not intended for diagnostic use.

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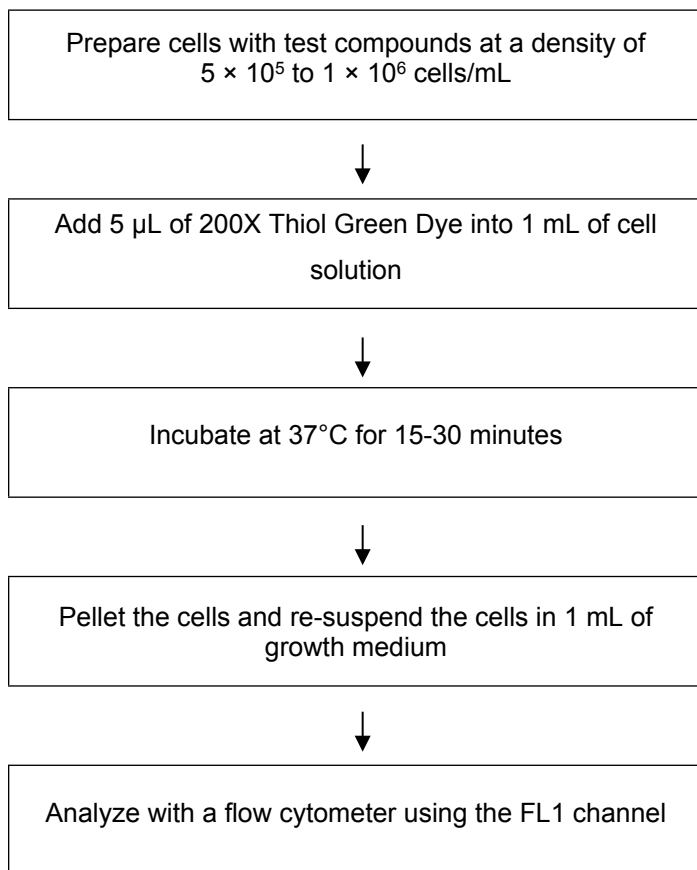
1. Introduction

There are a variety of parameters that can be used for monitoring cell apoptosis. ab112132 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.

ab112132 Intracellular GSH Assay Kit uses our proprietary non-fluorescent Thiol Green Dye, which becomes strongly fluorescent upon reacting with thiol (including GSH in cells). In normal cells, the Thiol Green Dye is accumulated primarily in cytosol, but it is partially translocated to mitochondria in apoptotic cells while Thiol Green Dye staining intensity is decreased. Cells stained with Thiol Green Dye can be visualized with a flow cytometer at Ex/Em = 490/520 nm (FL1 channel).

ab112132 can be used together with other reagents, such as 7-AAD for multi-parametric study of cell viability and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

2. Protocol Summary



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: Thiol Green Dye	1 x vial
Component B: Assay Buffer	1 x 100 mL
Component C: DMSO	1 x 0.5 mL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Assay Protocol

A. Preparation of 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.

B. Preparation of 200x Thiol Green Dye

Add 500 μ L of DMSO (Component C) into the vial of Thiol Green Dye (Component A), and mix well.

Note: Aliquot and stored the unused Component A at -20°C . Avoid repeated freeze/thaw cycles.

C. Run GSH Assay:

1. Treat cells with test compounds for a desired period of time to induce apoptosis.
2. Add 5 μ L of 200X Thiol Green Dye (from Step B), and incubate the cells in a 37°C , 5% CO_2 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 Thiol 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.** 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.
- 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.

6. Data Analysis

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

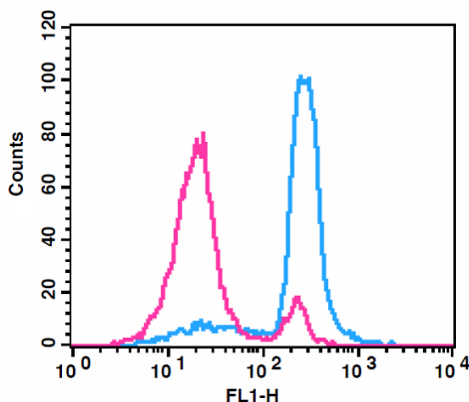


Figure 1. The decrease in the fluorescence intensity of the Thiol Green Dye with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated overnight without (blue line) or with 20 μM camptothecin (pink line) in a 37°C, 5% CO_2 incubator, and then dye loaded with Thiol Green Dye for 30 minutes. The fluorescence intensity of the Thiol Green Dye was measured using the FL1 channel.

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