

Intracellular Glutathione Assay Kit

Catalog# / Size	421936 / 1 kit
Regulatory Status	RUO
Other Names	GSH assay kit, glutathione sensor, GSH probe, GSH Live Cell Assay, Glutathione Kit
Description	The antioxidant reduced glutathione (GSH) plays an essential role in preserving a reduced intracellular environment, and protects cellular macromolecules like DNA, proteins and lipids against oxidizing agents. Reactive oxygen species can oxidize cellular GSH, leading to the loss of intracellular redox homeostasis and activation of the apoptotic signaling cascade. The Intracellular Glutathione Assay Kit is designed to measure GSH in live cells by flow cytometry and live-cell imaging. This fluorometric assay is based on the detection of GSH in cells using the GSH Probe Green, which is non-fluorescent in its native state but becomes strongly fluorescent upon reacting with glutathione. Fluorescence intensity decreases in cells with low GSH levels, as well as in cells undergoing apoptosis. This kit provides enough reagents for 100 tests (100 mL total working solution for up to 1×10^8 cells). A single test is defined as 1 mL of working solution for up to 1×10^6 cells.

Product Details

Verified Reactivity	Human
Formulation	• 1 vial (50 μL) of Glutathione Probe Green • 1 bottle (100 mL) of Glutathione Assay Buffer • 1 vial (500 μL) of DMSO
Storage & Handling	The kit should be stored at $< -15^{\circ}\text{C}$ protected from light.
Application	Live cell imaging - Quality tested FC - Verified
Application Notes	<u>Detection/Imaging Guidelines:</u> - Fluorescence microscope filter set: GFP/FITC filter set - Flow cytometry channels: • Ex/Em = 590/620 nm • Excitation: 488 nm laser • Emission: 530/30 nm filter • Instrument specification(s): FITC channel

Components and storage

This kit is stored at -20°C . For convenience, the buffer and DMSO can be stored at 4°C .
 - 1 vial of Glutathione Probe Green (50 μ L) (store at -20°C and minimize light exposure)
 - 1 bottle of Glutathione Assay Buffer (100 mL)
 - 1 vial of DMSO (500 μ L)

Required Materials Not Included:

- For live cell imaging: tissue culture vessel with coverslip bottom

Assay Protocol:

This kit provides enough reagents for 100 tests (for up to 1×10^8 cells). A single test is defined as 1 mL of Glutathione Probe Green working solution for up to 1×10^6 cells.

Preparation of 200X Glutathione Probe Green:

Bring DMSO and Glutathione Probe Green provided in the kit to room temperature. Add 500 μ L of DMSO into the vial containing 50 μ L of Glutathione Probe Green Stock Solution and mix well to make 200X Glutathione Probe Green. If not using immediately, aliquot the 200X Glutathione Probe Green and store at -20°C protected from light. Avoid freeze thaw cycles.

Experimental Protocol: 1. Bring all components of the kit to room temperature before starting. Treat your cell samples under desired experimental conditions in complete media. 2. Prepare the Glutathione Probe Green Working Solution by adding 5 μ L of the 200X Glutathione Probe Green to 1 mL media for up to 1×10^6 cells. For larger cell amounts, scale up accordingly. 3. Gently remove media

from your cells and add the Glutathione Probe Green Working Solution and incubate the samples at 37°C for 15-30 minutes. 4. **For live cell imaging:** Gently wash the cells 3 times with Glutathione Assay Buffer or buffer of choice and add the desired volume of Glutathione Assay Buffer or your buffer of choice for imaging. Observe cells with an inverted fluorescence microscope using the GFP/FITC filter set.

For flow cytometry: Spin down cells and resuspend in the desired volume of Glutathione Assay Buffer or your buffer of choice. Monitor the fluorescence intensity on a flow cytometer using the FITC (530/30 nm) channel.

Antigen Details

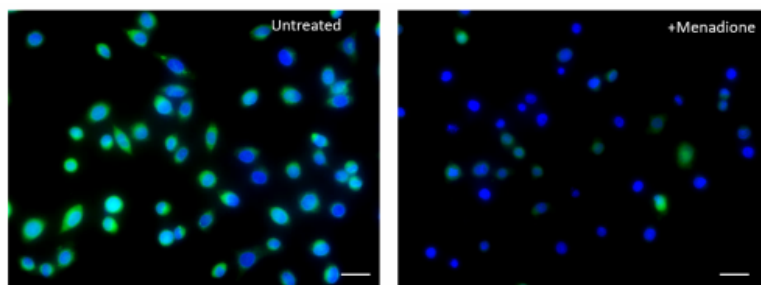
Distribution	Cytoplasm
Function	Reduced glutathione sensor
Biology Area	Cell Proliferation and Viability, Mitochondrial Function

Antigen References

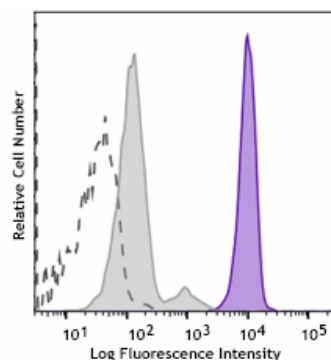
1. Maulucci G, *et al.* 2009. *Biosens Bioelectron.* 25:682-7.
2. Bellomo G, *et al.* 1997. *Microsc Res Tech.* 36:243-52.
3. Baylor KJ, *et al.* 1997. *Biochem Soc Trans.* 25:47S.
4. Jin T, *et al.* 2008. *Int J Mol Sci.* 9:2044-2061.

Gene ID NA

Product Data



Live cell fluorescence imaging of HeLa cells. HeLa cells were left untreated (left) or treated with 25 μ M Menadione (right) for 30 minutes. Menadione induces the formation of reactive oxygen species and depletion of GSH-mediated apoptosis. After treatment, cells were probed using the Intracellular Glutathione Assay Kit. In cells treated with Menadione, green fluorescence is reduced indicating a decrease in intracellular levels of reduced Glutathione. Images were captured using a 40X objective. Scale bar: 20 μ m



Jurkat cells were stained using the Intracellular Glutathione Assay Kit (purple histogram) or left unstained (dashed histogram). Dead cells (light gray histogram) have low levels of GSH and low fluorescence intensity.

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