# Intracellular GSH Assay

Catalog #9137

**FOR RESEARCH USE ONLY.**
Not for use in diagnostic procedures.

*Assess GSH in whole cells using flow cytometry*

## 1. INTRODUCTION

γ-L-glutamyl-L-cysteinyl-glycine (GSH; glutathione) is the most abundant non-protein thiol in cells. GSH is involved in the regulation of a number of essential biochemical processes within the cell. Primarily recognized as a key intracellular source of reducing power for combating the toxic accumulation of free radical by-products, GSH is also involved with detoxification and removal of exo/endogenous toxins and alkylating agents. In its role as a cell signaling agent, GSH is involved in DNA synthesis and cell proliferation regulation. Due to the genetically conserved molecular processes by which cells die, intracellular levels of GSH can favor a cell death pathway via apoptosis (adequate intracellular GSH stores) or via necrosis or autophagy (depleted intracellular GSH stores). Detection of a drop in intracellular GSH concentration in an experimental cell population relative to a negative (healthy cell) control is often indicative of an apoptosis induction event. Due to this strong correlation between intracellular GSH depletion and the onset of the apoptotic process, ICT's Intracellular GSH Assay provides a good screening option for assessing the potency of apoptosis inhibitor and activator reagents.

The kit provides all the essential reagents and an easy to follow protocol to assess changes in intracellular GSH levels using a flow cytometry analysis method. The key reagent in the assay is a proprietary thiol-sensitive dye, ThioBright™ Green. This thiol-reactive dye quickly penetrates cell membrane structures and accumulates primarily within the cytosol of living cells. In the presence of free-thiol-containing molecules such as GSH, the non-fluorescent ThioBright Green dye molecule binds covalently to the GSH target molecule and converts to the fluorescent form of the dye. During periods of oxidative stress or GSH depletion associated with cell death processes, cytosolic concentrations of the green fluorescent dye form become significantly diminished. The reduction in intracellular GSH concentration directly translates into an easily monitored reduction in the green fluorescence output within the dying or oxidatively stressed cell population. Because the GSH-bound form of ThioBright Green has the fluorescence properties of fluorescein (Ex/Em = 490/525 nm), it is perfectly suited to most standard flow cytometry instrument optical packages. Background fluorescence issues are minimized due to the non-fluorescent nature of the free form of the thiol-sensitive dye.

Intracellular GSH Assay requires minimal procedural steps and hands-on time to complete. Experimentally treated suspension or trypsin-EDTA disassociated adherent cells are ready for analysis after briefly staining (15-30 minutes) with the ThioBright Green GSH quantitation dye. Since the unbound reagent is non-fluorescent, subsequent wash steps are not required, thus simplifying the assay procedure.

Each kit will enable the assessment of up to 100 (1 mL) samples via flow cytometry. This flow cytometer assay can be adapted for use with a fluorescence microscope or plate reader equipped with FITC/FAM dye-compatible excitation/emission optics. Use and optimization of these alternative fluorescence analysis methods will require additional modifications to this flow cytometer based protocol.

## 2. KIT CONTENTS

- 1 vial of ThioBright™ Green, #6636
- 1 bottle Assay Buffer, 100 mL, #6637
- 1 vial of DMSO, 500 µL, #6638

## 3. STORAGE

The entire unopened kit should be stored frozen until the expiration date.

- Aliquot and store unused reconstituted ThioBright Green reagent at ≤ -20°C for up to 6 months. Avoid repeated freeze/thaw cycles.
- Assay Buffer may be stored frozen for up to 6 months.
- Protect ThioBright Green from light.

## 4. SAFETY DATA SHEETS (SDS)

Safety data sheets are available online at www.immunochemistry.com or by calling 1-800-829-3194 or 952-888-8788.

## 5. RECOMMENDED MATERIALS

- 15 mL polypropylene centrifuge tubes (1/sample)
- FACS tubes
- Cultured cells treated with the experimental conditions ready to be labeled

ImmunoChemistry TECHNOLOGIES LLC

**ImmunoChemistry Technologies, LLC**
9401 James Ave. S. #155, Bloomington, MN 55431 USA 800-829-3194
*immunochemistry.com*

- Reagents to induce oxidative stress, such as tert-Butyl hydroperoxide (e.g. Sigma catalog #458139), or apoptosis, such as staurosporine (catalog #6212) or camptothecin (catalog #6210)
- Hemocytometer
- Centrifuge at 200 x g

## 6. DETECTION EQUIPMENT

ThioBright 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 (catalog #6163), for multi-parametric study of cell viability and GSH levels.

## 7. EXPERIMENTAL PREPARATION & CONTROLS

Staining with ThioBright Green can be completed in 15-30 minutes. However, because it is used to label living cells, adequate time must be allotted for the cultivation of cell samples and the experimental treatment or apoptosis induction process. Allocate a sufficient amount of time for the experimental condition to manifest. The optimal exposure time will vary from several hours to several days depending upon the mechanism involved.

Cell concentrations used for analysis should be 5 x $10^5$ to 1 x $10^6$ cells/mL prior to labeling. Avoid stressful culture conditions; cell concentrations that are too high or too low can result in the creation of false positive artifacts and lead to erroneous interpretation of the data.

1. Culture cells to a density optimal for the specific experiment or apoptosis induction protocol. Cell density should not exceed $10^6$ cells/mL. Cells cultivated in excess of this concentration may begin to naturally enter apoptosis.
2. Create experimental and control cell populations:
   a. Treated experimental population: cells exposed to the experimental condition or treatment.
   b. Negative control: non-treated cells grown in a normal cell culture environment.
   c. Solvent vehicle control: cells grown in a normal cell culture environment spiked with an equivalent concentration of solvent used to dissolve the experimental compound; control 2b may be substituted if the solvent used has a low to non-discernible influence on cell viability.
   d. Positive control: cells induced to undergo apoptosis or oxidative stress event using a validated treatment method.

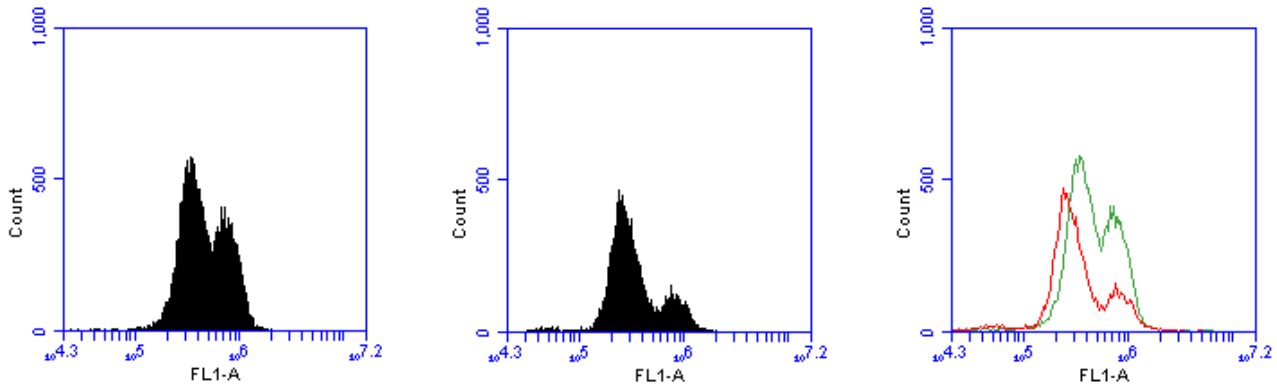
## 8. PREPARATION OF 200X THIOBRIGHT GREEN STOCK

ThioBright Green dye is supplied as a lyophilized powder that must be solubilized with DMSO to create a 200X stock concentrate. Each kit includes 1 vial of ThioBright Green GSH quantitation dye, which provides enough reagent to stain 100 samples (1 mL per sample) for flow cytometry analysis.

1. Thaw the kit and bring components to room temperature. Protect ThioBright Green from light and use gloves when handling all reagents.
2. Reconstitute each vial of ThioBright Green with 500 µL DMSO (included in kit) to form the 200X stock solution. Mix well. Once solubilized in DMSO, the stock solution will appear orange in color. Protect from light. Once reconstituted, the stock solution may be stored at ≤-20°C for 6 months, protected from light and thawed no more than twice during that time.

### FIGURE 1: ANALYSIS VIA FLOW CYTOMETRY

Jurkat cells were treated with a negative control (left histogram) or staurosporine, an apoptosis-inducing agent (middle histogram), for 4 hours at 37°C and then stained with ICT's Intracellular GSH Assay (catalog #9137) for 30 min. Cells were read on an Accuri C6 flow cytometer using an FL1 99% (2 log) attenuation filter. The median fluorescence intensity (MFI) of stained cells in the negative control was 425,971 in FL1-A (left: Negative, Non-Induced), whereas the treated population had a value of 289,169 (middle: Positive, Apoptosis-Induced), which is a decrease of more than 30%. The effect of staurosporine on intracellular GSH is easily visible when the samples are overlaid in a single plot (right: Overlay, green: Negative, red: Positive). Data courtesy of Ms. Tracy Hanson, ICT, 216:52, 051612.




Negative, Non-Induced | Positive, Apoptosis-Induced | Overlay

- **Warning!** Contains Dimethyl sulfoxide (DMSO), which is a combustible liquid, causes skin irritation, and causes serious eye irritation. In case of exposure, immediately flush eyes or skin with water. See SDS for further information.

## 9. STAINING PROTOCOL FOR SUSPENSION CELLS

1. Expose cells to the experimental condition and prepare control cell populations (Section 7). This can be done in the same cell culture flask, plate, or vessel used to cultivate the research model cell line.
2. Transfer cells into 15 mL polypropylene centrifuge tubes or FACS tubes to undergo ThioBright Green staining.
3. When ready to stain cells, spike the 200X ThioBright Green stock solution at a 1:200 v/v ratio directly into the culture media containing the cells. For example, add 5 µL ThioBright Green to each 995 µL sample. If larger samples are being used, add ThioBright Green at 1:200.
4. Incubate sample tubes for 15-30 minutes at 37°C in a $CO_2$ incubator. The optimal incubation time may vary with different cell lines. It is recommended that the incubation conditions be optimized within each laboratory to accommodate the varying cell model systems being utilized.
5. **Optional:** ThioBright Green-stained cells may be centrifuged and resuspended in Assay Buffer (included in kit) or other isotonic buffer prior to analysis (Section 11).

## 10. STAINING PROTOCOL FOR ADHERENT CELLS

1. Seed adherent cells into culture flasks or plates at a concentration that will eventually provide a monolayer confluency <80% at the end of the experimental treatment period. Different adherent cell lines expand at different growth rates. Each cell line model must be evaluated to assess the seeding volume and time to achieve an 80% (or less) monolayer confluency coverage prior to beginning the experiment.
2. Expose cells to the experimental condition and prepare control cell populations (Section 7). This can be done in the same cell culture flask, plate, or vessel used to cultivate the research model cell line.
3. Disassociate adherent cells from plate or flask surface:
   a. Remove cell culture media.
   b. Rinse with sterile PBS to help remove residual FBS (trypsin-neutralizing) components.
   c. Incubate monolayer with a sterile commercial product consisting of trypsin (0.05% w/v) + 0.5 mM EDTA. Some cell lines may be detachable using only 0.5 mM EDTA. Trypsin cleaves proteins that attach a cell to its substratum, while EDTA chelates the $Ca^{2+}$ and $Mg^{+}$ ions that facilitate cell adherence functionality. Thus, EDTA is less damaging than trypsin treatment.
   d. As soon as the cell monolayer has detached, neutralize trypsin (if used) to prevent harm to cells caused by excessive enzyme activity. Trypsin enzyme activity can be efficiently neutralized via natural trypsin inhibitor components present in fetal bovine serum (FBS). Simply resuspend the trypsin-EDTA detached cell monolayers into a 3-4 fold larger volume of cell culture media + 20% FBS.
   e. Pellet cell suspension by centrifuging at 200 x g. Remove supernatant and replace with the normal cell culture media formulation.
4. Stain cells by adding a 1:200 v/v dilution of the 200X ThioBright Green stock solution directly to the cell suspension cultures. For example, add 5 µL ThioBright Green to each 995 µL sample. If larger samples are being used, add ThioBright Green at 1:200.
5. Incubate sample tubes for 15-30 minutes at 37°C in a $CO_2$ incubator. The optimal incubation time may vary with different cell lines. It is recommended that the incubation conditions be optimized within each laboratory to accommodate the varying cell model systems being utilized.
6. **Optional:** ThioBright Green-stained cells may be centrifuged and resuspended in Assay Buffer (included in kit) or other isotonic buffer prior to analysis (Section 11).

## 11. ANALYZE DATA

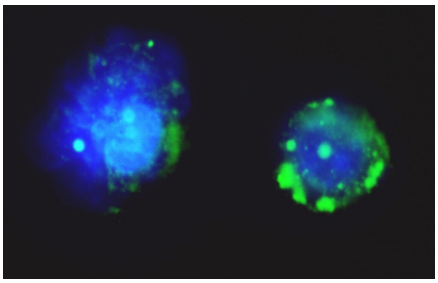
1. Monitor the fluorescence intensity of the ThioBright Green stained cell populations using flow cytometry. ThioBright Green exhibits fluorescence excitation and emission characteristics similar to FITC or FAM fluorophores. It is efficiently excited (490 nm optimal) using standard blue laser optics packages, which are present on most flow cytometer instruments, and it is easily detected using typical FL1 green-emission-channel optics (530/30 nm).
2. Create a FSC-A vs SSC-A dot plot, and then gate on the cells of interest. Plot FL1 emission intensity (X-axis) versus cell number (Y-axis) as a histogram. Compare the median fluorescence intensity (MFI) output generated within the negative control cell population to that observed in the positive (GSH depletion) control and experimental treatment cell populations. Determine if the solvent vehicle used to deliver the experimental compound (if applicable) had a significant influence on the relative intracellular GSH content.
3. In general, the lower the ThioBright Green-associated fluorescence output relative to the fluorescence output of the healthy cell negative control, the greater the degree of GSH depletion in those cell treatment groups. This assay is not designed to determine which particular cell pathology triggered the drop in intracellular GSH concentration in the treatment cell populations. Other testing parameters must be analyzed to establish the specific causation process responsible for the reduced intracellular GSH levels.

**RELATED PRODUCTS:**

To detect the intracellular process of apoptosis via activated caspases, use our FLICA® kits. These caspase assays are available in green, red, or far-red fluorescence for the preferred detection of individual active caspase enzymes or pan-caspase activity.

Visit www.immunochemistry.com for more information.

- Label the active apoptotic process
- Distinguish apoptosis from necrosis
- Whole cell analysis
- Counterstain with common fluorophores.



**ABOVE:** Apoptotic neuroblastoma cells fluoresce green after staining with FAM-FLICA® Poly Caspase Assay (#92). Hoechst 33342 (blue in image) nucleic acid stain is included in the kit as well as Propidium Iodide live/dead stain (not shown).


**ABOVE:** SR-FLICA® Poly Caspase Assay, Standard Size (#917)

**FOR RESEARCH USE ONLY.**
Not for use in diagnostic procedures.

*Thank you for using our Intracellular GSH Assay! If you have any questions, or would like to share your data, please contact us at 1-800-829-3194 or 952-888-8788, or send an email to help@immunochemistry.com.*

ImmunoChemistry TECHNOLOGIES LLC

**BRIGHT MINDS, BRIGHT SOLUTIONS.**

ImmunoChemistry Technologies, LLC gratefully acknowledges the significant contributions made by one of its founders, Brian W. Lee, Ph.D in the development of this product, including the creation and illustration of its strategy and protocol.

**Immunochemistry Technologies, LLC**
9401 James Ave. S. #155, Bloomington, MN 55431 USA 800-829-3194
immunochemistry.com