Product Information

Glutathione Fluorometric Assay Kit

I. Kit Contents:

Components	K2098-100 100 assays	Cap Color	Part Number
Cell Lysis Buffer	25 ml	WM	K2098-C-1
Monochlorobimane	200 µl	Red	K2098-C-2
GST Reagent	200 µl	Green	K2098-C-3
GSH Standard (1 mg; MW 307)	1 Vial	Yellow	K2098-C-4

II. Introduction:

Glutathione is a low-molecular-weight intracellular thiol. It is essential for protecting mammalian cells from oxidative and nitrosative stress. In the early stages of apoptosis, abolished glutathione levels was observed. The Glutathione Fluorometric Assay Kit gives a simple in vitro assay for detecting total glutathione changes in apoptosis and other samples. This kit applies a dye called monochlorobimane (MCB) that forms an adduct with glutathione. When MCB bound to glutathione of reduced or oxidized form, the dye emits blue fluorescence (Ex/Em = 360 nm/461 nm). The reaction is catalyzed by glutathione S-transferase and the level of total glutathione can be detected by fluorometer or fluorometric plate reader.

III. Reagent Preparations:

A. General Consideration and Reagent Preparations:

1. Allow reagents to warm to room temperature and briefly centrifuge vials prior to opening. Read the entire protocol before the assay.

2. After opening the kit, store MCB, GST, GSH at -20℃. Store Cell Lysis Buffer at 4℃.

3. Monochlorobimane is dissolved in DMSO and needs to be warmed > 18℃ prior to use (usually 1 – 2 min in a 37℃ water bath followed by a brief centrifugation is sufficient).

4. Reconstitute the GSH Standard with 100 µl dH2O to generate a 10 µg/µl standard stock solution. Freeze immediately after each use.

B. Sample Preparation:

1. Apoptosis Assay Samples: Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.

2. Cells or Tissues: Collect cells (1 x 10^6) into 1.5 ml microcentrifuge tubes, centrifugation at 700 x g for 5 minutes, carefully remove the medium. Lyse the cell pellets or 10 mg tissue in 100 µl Cell Lysis Buffer. Incubate on ice for 10 minutes, then centrifuge at top speed in a tabletop centrifuge for 10 minutes. Transfer the supernatant into new tubes for glutathione assay.

3. Liquid Samples: Assay directly or dilute with Cell Lysis Buffer.

Note: If proteins or enzymes are believed to interfere with the assay, samples can be deproteinated by centrifugation through a 10 kDa molecular weight cut off filter before performing the assay.

IV. Glutathione Assay Protocol:

1. Standard Curve Preparation: Dilute 10 µl of the reconstituted 10 µg/µl Standard GSH stock solution into 990 µl Cell Lysis Buffer to generate 0.1 µg/µl Standard GSH solution (use a fresh dilution each time, and use immediately), mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells of a 96-well plate to generate 0; 0.2; 0.4; 0.6; 0.8; 1.0 µg/well GSH standard. Bring total volume to 100 µl with Assay Buffer for each well.

2. Samples: Add different volumes of sample directly into 96-well plate. Bring total volume to 100 µl with Assay Buffer. For unknown samples, we suggest including several dilutions for each sample so that the reading will be within the standard curve range. In our experience using HeLa cells prepared in this manner, 20 µl appeared to be the optimum amount of sample.

3. Reaction: Add 2 µl of the GST Reagent and 2 µl of MCB into each sample and standard. Mix the plate well. Incubate the plate at 37℃ for 1 hour. It can be very informative to acquire the fluorescence data kinetically during the incubation to observe the GSH-MCB adduct formation.

4. Measurement: Measure the fluorescence value in a fluorometer or fluorescence plate reader at Ex./Em. = 360 ± 20 nm/460 ± 20 nm.

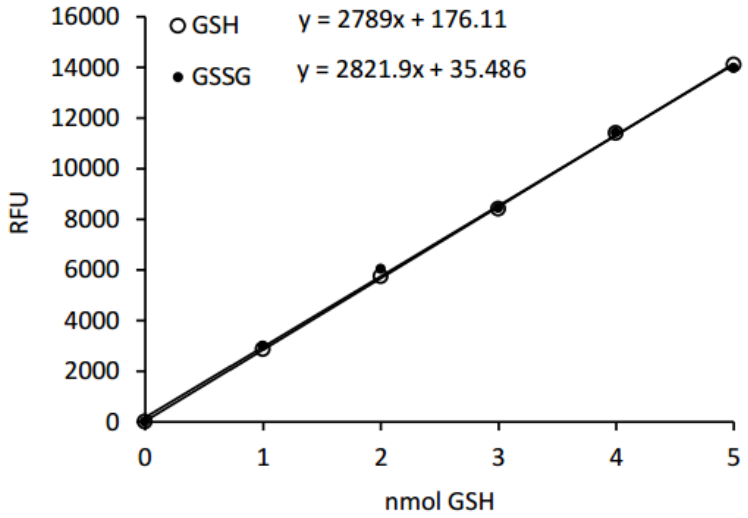
5. Calculation: Subtract 0 standard reading from all readings. Plot Standard Curve. Apply sample readings to the standard curve to calculate total glutathione amount in each sample well. The glutathione concentration in sample can be calculated as follows:

C = A/V µg/ml

Where A: The total glutathione amount from standard curve (in µg).

V: Original sample volume added into sample well (in ml).

The results can be expressed as µg/ml of sample, µg/10^6 cells, or for apoptosis assay, as the percentage change in glutathione level in treated samples vs untreated control samples. Reduced Glutathione molecular weight: 307 g/mol.



General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample	• Refer data sheet for details about incompatible samples • Use the reaction buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes);

	• Use of old or inappropriately stored samples	observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and re-suspend all components before preparing the reaction mix • Avoid pipetting small volumes • Pipette gently against the wall of the tubes • Prepare a master reaction mix whenever possible • Always refer the dilutions in the data sheet • Use fresh components from the same kit • Recheck calculations after referring the data sheet
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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