ApoGSH™ Glutathione Detection Kit

(Catalog #K251-100; 100 assays; Store kit at –20°C)

I. Introduction:

Glutathione is the principal intracellular low-molecular-weight thiol that plays a critical role in the cellular defense against oxidative and nitrosative stress in mammalian cells. Diminished glutathione levels have been observed in the early stages of apoptosis. BioVision's ApoGSH™ Glutathione Detection Kit provides a simple *in vitro* assay for detecting total glutathione changes during apoptosis and other conditions. The assay utilizes monochlorobimane (MCB), a dye that forms an adduct with glutathione. The unbound MCB is almost nonfluorescent, whereas the dye fluoresces blue (Ex/Em = 380/461 nm) when bound to glutathione. The reaction is catalyzed by glutathione S-transferase. The assay detects both reduced and oxidized glutathione.

II. Kit Contents:

Components	100 Assays	Cap Color	Part Number
Cell Lysis Buffer	25 ml	WM	K251-100-1
Monochlorobimane	200 µl	Red	K251-100-2
GST Reagent	200 µl	Green	K251-100-3
GSH Standard (1 mg; MW 307)	1 Vial	Yellow	K251-100-4

III. Glutathione Assay Protocol:

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°C. Store Cell Lysis Buffer at +4°C.
3. Monochlorobimane is dissolved in DMSO and needs to be warmed > 18°C prior to use (usually 1 – 2 min in a 37 °C water bath followed by a brief centrifugation is sufficient).
4. Reconstitute the GSH Standard with 100 µl dH_2O 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 (BioVision, Cat #1997-25) before performing the assay.

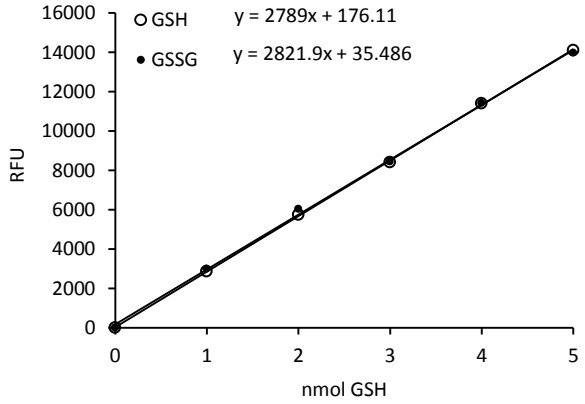
C. Assay Protocol:

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. For additional understanding of how this assay was developed and used with HeLa cells go to our website and open **TECH NOTE K251**.
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°C 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.



RELATED PRODUCTS:

- Glutathione Colorimetric Assay Kit;
- Glutathione (GSH, GSSG, total) Assay Kit
- Peroxidase Assay Kit
- Glutathione Sepharose Beads
- GST Colorimetric and Fluorometric Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		