BioVision

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For research use only

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 detection of total glutathione changes in apoptosis and other samples. The assay utilizes monochlorobimane (MCB), a dye that appears to form an adduct exclusively with glutathione. The unbound MCB is almost nonfluorescent, whereas the dye fluoresces blue (Ex./Em. = 380nm/461nm) when bound to glutathione of reduced or oxidized form. The reaction is catalyzed by glutathione S-transferase. Thus, the amount of total glutathione can be easily detected using a fluorometer or a 96-well fluorometric plate reader.

II. Kit Contents:

Components	100 Assays	Cap Color	Part Number
Cell Lysis Buffer	25 ml	WM	K251-100-1
Monochlorobimane (25 mM)	200 µl	Red	K251-100-2
GST Reagent (50 U/ml)	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:

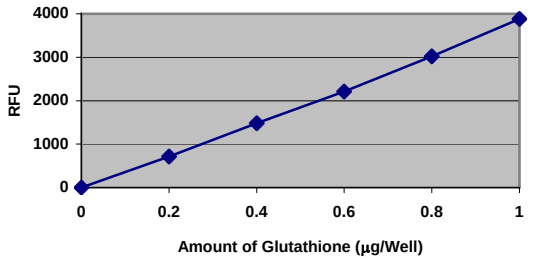
1. Monochlorobimane was dissolved in DMSO, need to be warmed >18°C when use.
2. After opening the kit, store MCB, GST, GSH at -20°C. Store Cell Lysis Buffer at 4°C for conveniently use.
3. Reconstitute the GSH standard with 100 µl dH2O to generate 10 µg/µl standard stock solution. Freeze immediately after each use.

B. Sample Preparation:

1. **Apoptosis Assays:** Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction. Collect cells (1 x 10^6) into 1.5 ml microcentrifuge tubes. Centrifugation at 700 x g for 5 minutes, carefully remove the supernatant. Lyse the cell pellets in 100 µl ice-cold Cell Lysis Buffer. Incubate on ice for 10 minutes, then centrifuge at top speed in an eppendorf centrifuge for 10 minutes. Transfer the cell lysate into labeled new tubes for glutathione assay.
2. **Cells or Tissues:** Lyse 1x 10^6 cells or 10 mg tissues in 100 µl Cell Lysis Buffer as described above (B. 1).
3. **Liquid Samples:** Assay directly or dilute with Cell Lysis Buffer.
4. If proteins or enzymes are the concern to interfere with the assay, samples can be deproteinized by spin samples through 10Kd molecular weight cut off filter (BioVision, Cat #1997-25) before 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 fresh dilute each time), mix well. Add 0, 2, 4, 6, 8, 10 µl into each individual wells of a 96-well plate to generate 0; 0.2; 0.4; 0.6; 0.8; 1.0 µg/well glutathione standard. Add Cell Lysis Buffer to a total volume of 100 µl for each well.
2. **Samples:** Dilute assay samples with Cell Lysis Buffer to total volume 100 µl in the 96-well plate. For unknown sample, we suggest to include several dilutions for each sample, so that the results will be in the standard curve range.
3. **Reaction:** Add 2 µl of the 50 U/ml GST Reagent and 2 µl of MCB dye into each samples and standards separately. Mix the plate well. Incubate the reaction at 37 °C for 30 min.
4. **Reading:** Measure the fluorescence value in a fluorometer or fluorescence plate reader at Ex./Em. = 380/460 nm. Apply the sample readings to the standard curve to calculate total glutathione amount in each sample. The results can be expressed by ng/ml of sample; or ng/10^6 cells; or for apoptosis assay: percentage of glutathione level of untreated control cells.



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