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Biotium, Inc.
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MCB Glutathione Detection Kit

Catalog Number: 30019 (100 assays)

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Description

Diminished cellular glutathione (GSH) level occurs at the early stage of mitochondria associated apoptosis pathway due to GSH efflux. GSH depletion further leads to cytochrome c release and caspase 3 inductions. Glutathione Fluorometric Detection Kit utilizes monochlorobimane (MCB), a dye that has a high affinity for GSH for GSH detection [1-4]. The unbound dye is almost nonfluorescent, wheras the thiol-bound dye fluoresces blue (excitation=380 nm; emission=461 nm). By incubating cellular lysate with MCB, the intensity of the fluorescence signal generated from the assay reflects the amount of GSH present in the cells. The reaction is catalyzed by glutathione S-transferase. Therefore, fluorescence can easily be detected after 30 min incubation using a fluorometer or 96-well fluorometric plate reader.

Kit Components

25 mL Cell Lysis Buffer
500 uL MCB (10 mM)
200 uL GST Reagent (50U/mL)

Storage and Shelf Life

Store the kit at -20°C until use. The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.

Glutathione Assay Protocol

Note: *The following protocol was optimized using Jurkat cells. Other cell types in which glutathione levels drop during apoptosis may be used. However, the condition may need to be optimized.*

1. Induce apoptosis according to your specific protocol. Concurrently incubate a control culture without induction.
2. Collect cells (>1x10^6) by centrifugation at 700 x g for 5 minutes.
3. Remove supernatant and resuspend cell pellet in 1 mL ice-cold PBS.
4. Transfer into a 1.5 mL microcentrifuge tube, and centrifuge at 700 x g for 5 minutes at 4^{o}C. Remove supernatant.
5. Resuspend cells in 100 uL ice-cold Cell Lysis Buffer.
6. Incubate on ice for 10 minutes, then centrifuge at top speed in an eppendorf centrifuge for 10 minutes.
7. Transfer supernatant to a fresh tube or to a well on a 96-well plate.
8. Add 5 uL of the 10 mM MCB and 2 uL of the 50 U/mL GST Reagent.
   Note: *Prepare a negative control sample with 100 uL Cell Lysis Buffer, 5 uL MCB and 2 uL GST.*
9. Incubate all samples at 37^{o}C for 15-30 minutes.
10. Measure fluorescence in a fluorometer or fluorescence plate reader at Ex./Em. = 380/460 nm.

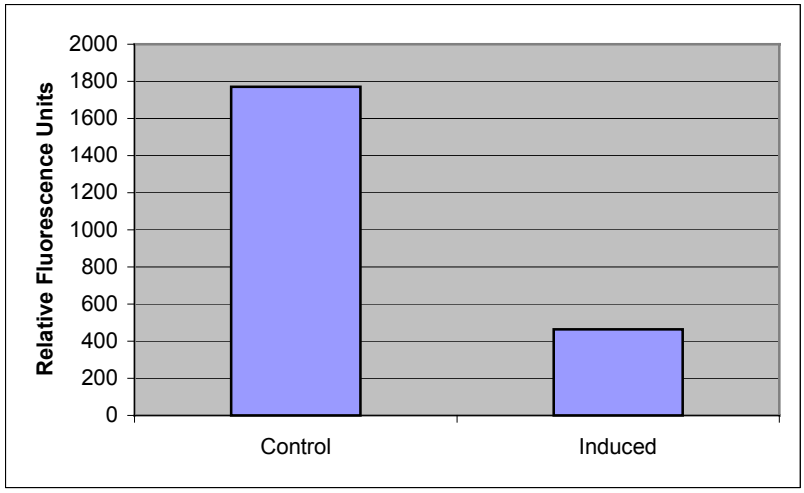

Figure 1. Diminished Glutathione Level in Apoptotic Cells. Jurkat cells were treated with DMSO (Control) or 1 uM staurosporine (Induced) for 4 hours. Glutathion level was measured using Biotium's MCB Glutathione Detection Kit. Fluorescence was measured using Ex./Em. = 380/460 nm.

References

1. Anal Biochem. 286(1):35 (2000)
2. Biochem. Soc. Trans. 28, 56 (2000).
3. FASEB J. 12(6), 479 (1998).
4. J Biol Chem. 263(28):14107 (1988)