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Product Information

MCB Glutathione Detection Kit

Catalog Number: 30019

Unit Size: 100 assays

Kit Contents

Component	Size
30019A: Cell Lysis Buffer	25 mL
30019B: 10 mM MCB	500 uL
30019C: GST Reagent	200 uL

Storage and Handling
Store at -20°C. Product is stable for at least 6 months from date of receipt when stored as recommended.

Spectral Properties
Ex/Em: 394/490 nm

Product Description
Diminished cellular glutathione (GSH) level occurs at the early stage of mitochondria associated apoptosis pathway due to GSH efflux. The MCB Glutathione Detection Kit utilizes monochlorobimane (MCB), a dye that has a high affinity for GSH (1-4). The unreacted dye is almost non-fluorescent. After glutathione-S-transferase (GST)-catalyzed reaction of the dye with GSH, the dye fluoresces blue (excitation/emission=394/490 nm) (Figure 1). By incubating cellular lysate with MCB and GST, the intensity of the fluorescence signal generated from the assay reflects the amount of GSH present in the cells (Figure 2).

Assay Protocol

The following protocol was developed using Jurkat suspension cells, and may be adapted for use with for other cell types.

1. Induce apoptosis according to your specific protocol. Remember to incubate a control culture without induction.
2. Transfer cells (>1x10^6) to a centrifuge tube and pellet by centrifugation at 700 x g for 5 minutes.
3. Remove supernatant and resuspend cell pellet in 1 mL ice-cold PBS.
4. Centrifuge at 700 x g for 5 minutes at 4°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 a microcentrifuge for 10 minutes.
7. Transfer the supernatant to a fresh tube or to a well in a 96-well plate.
8. Prepare negative control samples by adding 100 uL Cell Lysis Buffer to a tube or plate. Optional: 10 mM reduced glutathione in Cell Lysis Buffer can be used as a positive control.
9. Add 5 uL of 10 mM MCB and 2 uL of GST Reagent to each sample and mix well.
10. Incubate all samples at 37°C for 15-30 minutes.
11. Measure fluorescence in a fluorometer or fluorescence plate reader at Ex/Em = 394/490 nm.

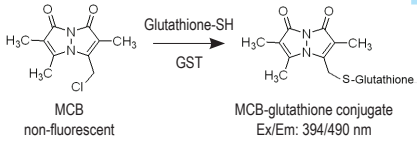

Figure 1. MCB glutathione assay principle.

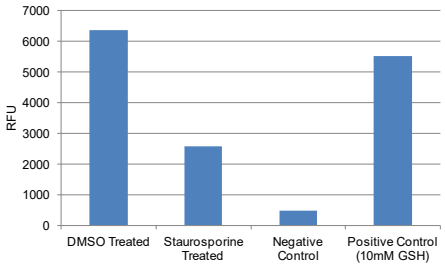

Figure 2. Jurkat cells were treated with 2 uM staurosporine for 4 hours to induce apoptosis, or with an equivalent volume of DMSO as a control, then assayed using the MCB Glutathione Detection Kit. Cell Lysis Buffer was used as a negative control, and 10 mM reduced glutathione (GSH) in Cell Lysis Buffer was used as a positive control. Fluorescence was read in a fluorescence microplate reader.

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).

Related Products

Catalog number	Product
70055	MitoView™633
70054	MitoView™ Green
30001	JC-1 Mitochondrial Membrane Detection Kit
30029	NucView™ 488 Caspase-3 Assay Kit for live cells
30067	Dual Apoptosis Assay Kit with NucView™ 488 caspase-3 substrate and CF™594-Annexin V
30062	NucView™488 and MitoView™633 Apoptosis Assay Kit
30072	NucView™488 and RedDot™2 Apoptosis & Necrosis Kit
30065	Apoptosis & Necrosis Quantitation Kit Plus
30066	Apoptotic, Necrotic & Healthy Cells Quantitation Kit Plus
30060	CF™488A Annexin V and 7-AAD Apoptosis Kit
30061	CF™488A Annexin V and PI Apoptosis Kit
30063	CF™488A TUNEL Assay Apoptosis Detection Kit
30064	CF™594 TUNEL Assay Apoptosis Detection Kit

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