FT-BU1410

Glutathione detection

Product Information

Name :	**mBCl Glutathione detection kit**		
Catalog Numbers:	mBCl Glutathione detection kit	FP-BU1410	100 tests
	Monochlorobimane, stand alone product	FP-38980A	25 mg
CAS	76421-73-3		
Molecular Formula	$C_{10}H_{11}ClN_2O_2$		
MW	226.66		
$\lambda_{Exc./Em.}$	380 / 460 nm		
Kit components:			
	Cell lysis buffer	25 ml	
	Monochlorobimane	200 µl (10mM)	
	GST positive control	500 µl (50U/ml)	

Storage: Store at -20°C (6 months)

Introduction

Diminished cellular glutathione (GSH) level occurs at the early stage of mitochondrion-associated apoptosis pathway due to GSH efflux. GSH depletion further leads to cytochrome c release and caspase 3 induction.
In the kit, a thiol-reactive dye monochlorobimane (mBCl) is used. It is essentially nonfluorescent until it reacts with a thiol to form a blue fluorescent product ($\lambda_{abs}/\lambda_{em}$ = 380/460 nm).
The incubation of cellular lysate and mBCl generes an intensity of the fluorescent signal and allows to give the amount of GSH present in the cells.
A fluorometer or 96- well fluorometric plate reader is used to detect it.

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 4ºC. 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 37ºC for 15-30 minutes.
10. Measure fluorescence in a fluorometer or fluorescence plate reader at Ex./Em. = 380/460 nm.

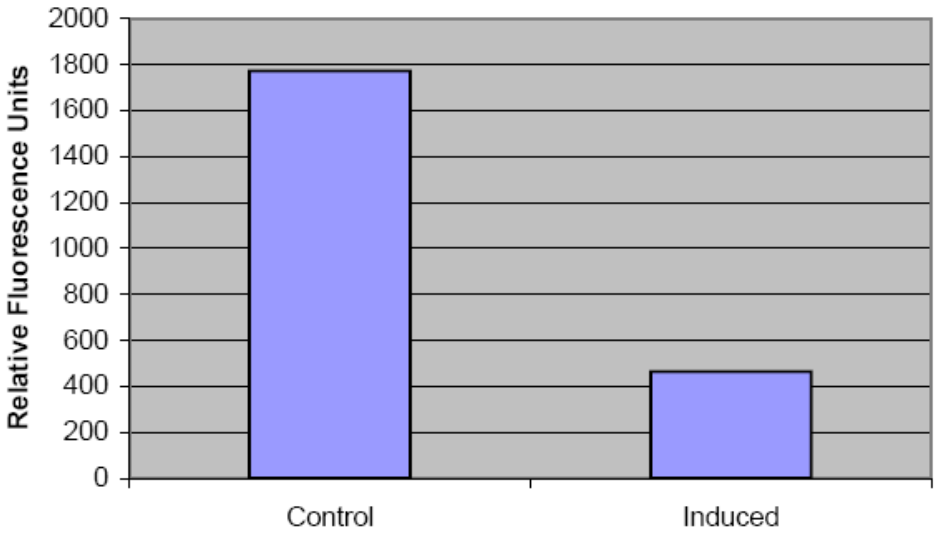

Figure 1. Diminished Glutathione Level in Apoptotic Cells. Jurkat cells were treated with DMSO (Control) or 1 uM staurosporine (Induced) for 4 hours. Glutathion level was measured using Biotium's MCB Glutathione Detection Kit. Fluorescence was measured using Ex./Em. = 380/460 nm.

References

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5**DeCory H**. *et al*., Efflux of Glutathione Conjugate of Monochlorobimane from Striatal and Cortical Neurons, *Drug Metabolism and Disposition*, Vol. 29, Issue 10, 1256-1262 (2001) Article
6**Franco R**. *et al*., Glutathione Depletion Is Necessary for Apoptosis in Lymphoid Cells Independent of Reactive Oxygen Species Formation, *J. Biol. Chem*., Vol. 282, Issue 42, 30452-30465 (2007) Article
7**Li S**. *et al*., Pro-oxidant effect of transforming growth factor-β1 mediates contractile dysfunction in rat ventricular myocytes, *Cardiovascular Research* 77(1):107-117 (2008) Article
8**Sun X**. *et al*., Two-photon Imaging of Glutathione Levels in Intact Brain Indicates Enhanced Redox Buffering in Developing Neurons and Cells at the Cerebrospinal Fluid and Blood-Brain Interface, *J. Biol. Chem*., Vol. 281, Issue 25, 17420-17431 (2006) Article

Related Products

- Live Cell Glutathione Transferase Activity Kit, BQ2350

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
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