



Cell Technology, Inc

Fluoro Thiol

Fluorescent Thiol Detection Kit

Key Benefits

- Detection of Reduced thiols in cells or tissue extracts.
- Sensitive fluorescent assay.
- Detection of reduced thiol levels in apoptosis, metabolism and oxidative stress.
- Diverse: detection of reduced thiols in Bacterial, fungal, plant cells or other samples.

Assay Principle

During the normal course of metabolism, oxygen is partly reduced as electrons leak out of the electron transport chain during respiration. These partially reduced oxygen species (ROS) can react with organic substances through non-catalytic means. Furthermore, ROS can be generated via endogenous enzyme systems like plasma NADPH oxidase, cytoplasmic xanthine oxidase and organelle sources e.g., cytochrome P-450. ROS have been implicated in regulating diverse cellular functions including proliferation, defense against pathogens, intra-cellular signaling, transcriptional activation and apoptosis. Elevation of ROS beyond the buffering capacity of the cell can lead to oxidative stress. Elevated ROS levels can lead to damage of DNA/RNA, proteins and lipids which may lead to apoptosis. Cells have developed several mechanisms to counter act elevated ROS levels such as a thiol reducing buffer composed of cellular thiol levels (glutathione and thioredoxin) for the maintenance of the reduction-oxidation (redox) state of the cell, and enzymes to remove ROS (catalase, superoxide dismutase and glutathione peroxidase) (1-2).

Reaction:

Cell Technology's Fluoro- Thiol kit detects Thiol levels in cells and tissue extracts. Hatsuo Maeda and co workers have developed 2,4-Dinitrobenzenesulfonyl fluorescein (dye) as a fluorescent specific probe for general thiol detection ⁽³⁾. The reaction scheme is outlined below.

Reaction:

1. Thiol (reduced) + non-fluorescent Dye \longrightarrow fluorescent analog excitation at 488nm and emission at 515-530nm.

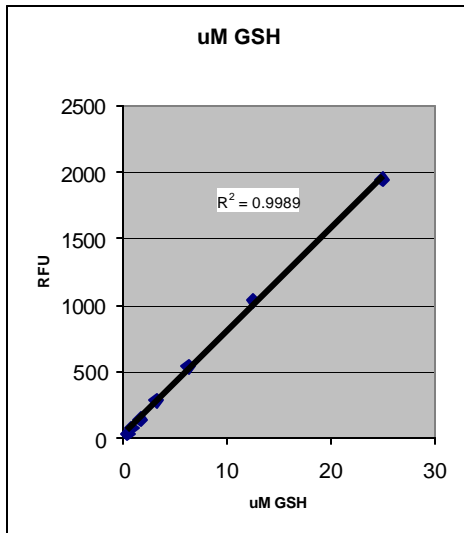


Figure 1. Detection of glutathione (GSH) utilizing the Fluoro Thiol kit.

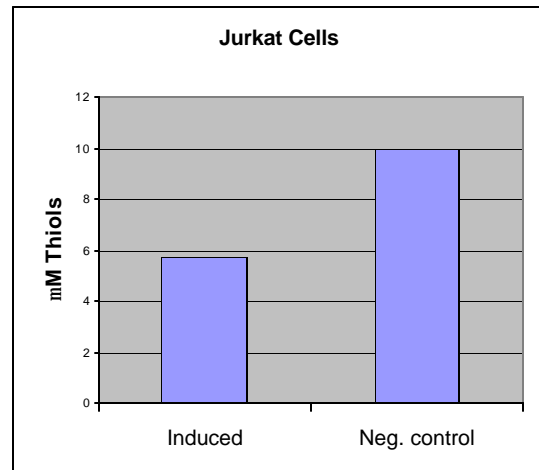


Figure 2. Jurkat cells were incubated with 1 μ M staurosporine (4) for 3 hours. After which thiol levels were quantified using Cell Technology's Fluoro Thiol kit. The graph represents approximately 1×10^4 cells per reaction (n=3).

Ordering Information

Catalog #	Size (Tests)	Price (US\$)
FLTHIO 100-2	100	395

References:

1. Gamaley IA and Klyubin IV (1999) Roles of reactive oxygen species: Signaling and regulation of cellular functions. *Int Rev Cytol* **188**:203–238. .
2. Nakamura H, Nakamura K and Yodoi J (1997) Redox regulation of cellular activation. *Annu Rev Immunol* **15**:351–369.
3. 2,4-Dinitrobenzenesulfonyl Fluoresceins as Fluorescent Alternatives to Ellman's Reagent in Thiol-Quantification Enzyme Assays*. Hatsuo Maeda,*Hiromi Matsuno,Mai Ushida, Kohei Katayama,Kanako Saeki,and Norio Itoh. *Angew.Chem.Int.Ed.*2005 ,44 ,2922 –2925
4. Marchetti,P.,*et al.* ,Redox regulation of apoptosis: impact of thiol oxidation status on mitochondrial function.*Eur.J.Immunol.*,**27** ,289-296 (1997).

Cell Technology, Inc.
950 Rengstorff Ave Suite D
Mountain View, CA 94043
USA

Tel: 650-960-2170 / 888-7ASSAYS
Fax: 650-960-0367
sales@celltechnology.com

www.celltechnology.com