Fluoro Thiol

Fluorescent Thiol Detection Kit

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Notes:

CELL TECHNOLOGY

Introduction

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 thioredoxion) for the maintance of the reduction-oxidation (redox) state of the cell, and enzymes to remove ROS (catalase, superoxide dismutase and glutathione peroxidase) (1-2).

Applications:

- 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.

I. Assay Principle:

Cell Technology's Fluoro Thiol kit detects reduced 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 reduced thiol detection [3]. The reaction scheme is outlined below.
Reaction:

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

II. Storage:

1. The kit should be stored at 2-8^{0}C until first use.

III. Warnings and Precautions:

1. **For Research use only. Not for use in diagnostic procedures**.
2. Practice safe laboratory procedures by wearing protective clothing and eyewear.
3. Keep the dye protected from direct lab lighting.
4. Avoid contact with reducing agents.

IV. Catalog # FLTHIO 100-2 Kit contents (for 100 assays):

1. **Part # 4021: Dye:** 1 vial dried. Upon arrival store at 2-8^{0}C.
2. **Part # 3053: Lysis buffer:** 1 bottle 25mL. Upon arrival store at 2-8^{0}C.
3. **Part# 7015: GSH Standard:** 3 vials dried. Upon arrival store at 2-8^{0}C.

Materials required but not supplied:

1. Black 96-well plates (clear bottom optional for bottom reading instruments).
2. Fluorescence plate reader.
3. Acetonitrile: Sigma Cat# 271004.

V. Preparation of reagent working solutions and Storage:

1. **Part # 4021. Dye**. Reconstitute the vial with 100µL of Acetonitrile**.** This will make a 50X solution. Aliquot into single use vials and store at –20^{0}C or below. After reconstituted the dye is stable of 6 months if stored accordingly.
   Note: Store in a manual defrost freezer.

2. **Part # 3053. Cell Lysis buffer:** Ready to use. Store at 2-8^{0}C.

3. **Part# 7015: GSH Standard**. You are provided with three vials of standard. Once reconstituted they should be used immediately. Do not store the reconstituted standards. Reconstitute the vial with 100uL of lysis buffer. This will make a 1mM solution.

VII. Mammalian Cell Preparation.

After your experimental procedure wash 2 x 10^5 – 1 x 10^6 cells twice with 2 mL of PBS. After the final wash decant the supernatant, gently vortex the cell pellet and add 200 µL of the cell lysis buffer (part# 3053). Centrifuge cell lysates for five minutes at 8000 to 10,000 x g to clarify supernatant. Supernatants should immediately be run in the assay. *Note: Each investigator should optimize the number of cells used per test.*

VIII. Tissue Preparation

Tissue preparation: Prior to tissue extraction exsanguinate (optional) the animal to remove red blood cells from tissue. Weigh 5-20 mg of tissue and rinse in ice cold PBS**.** Transfer tissue section to a 1.5 mL eppendorf tube and add 200µL of the cell lysis buffer (part# 3053). Then using standard techniques homogenize the tissue sample. Centrifuge tissue sample for five minutes at 8000 to 10,000 x g to clarify supernatant. Supernatants should immediately be run in the assay.

Note: Each investigator should optimize the concentration of homogenates used per test.

VIII. Assay Protocol:

1. **Make the Reaction cocktail**
   To each 1 mL of cell lysis buffer (Part # 3053) add 20 µL of the reconstituted dye (part# 4021). This is enough for 20 tests. Make enough Reaction cocktail for one day's worth of experiments.

   Note: Make the reaction cocktail right before use. Light Sensitive. Avoid direct laboratory light as this will increase background.

2. **GSH Standard Curve.**
   Label suitable tubes 1-8. To tube #1 add 475 µL of cell lysis buffer (Part# 3053) and 25µL of the reconstituted GSH standard.. This will make a 50µM solution of GSH. Next serially dilute (1:2) the 50µM GSH standard in cell lysis buffer (Part# 3053) to construct a standard curve. This can be accomplished by adding 250 µL of cell lysis buffer into tubes #2-8. From tube #1 remove 250 µL of the 50µM GSH standard and add it to tube #2. Gently vortex tube #2 and pipette out again 250 µL from tube#2 and add it to tube#3. Continue this process to tube #7. Tube # 8 is the blank control. The final GSH concentration in the well will be two times less than in the tube.

Tube #	GSH Concentration in tubes.	Final GSH Concentration in wells.
1	50 µM	25 µM
2	25 µM	12.5 µM
3	12.5 µM	6.25 µM
4	6.25 µM	3.125 µM
5	3.125 µM	1.56 µM
6	1.56 µM	0.781 µM
7	0.781 µM	0.390 µM
8	0	0

Add 50µL of standard or sample in triplicate to individual wells of a black 96 well plate. It is recommended to titrate out the sample in the cell lysis buffer several fold so it's values will fall within the range of the standard curve. Next pipette in 50µL of the reaction cocktail, from step 1 above, to all the wells. Incubate at room temperature in the **dark** for 15-20 minutes. Take a reading with excitation at 488 nm and emission at 515-530nm. Depending on the sensitivity of your instrument you maybe able to take readings with 10 minutes.

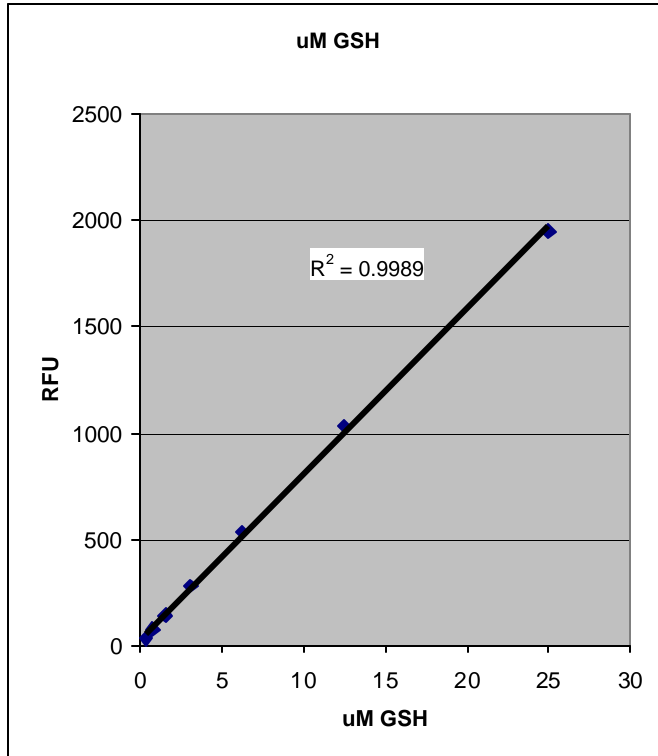

Figure1.Detection of glutathione (GSH) utilizing the Fluoro Thiol kit.

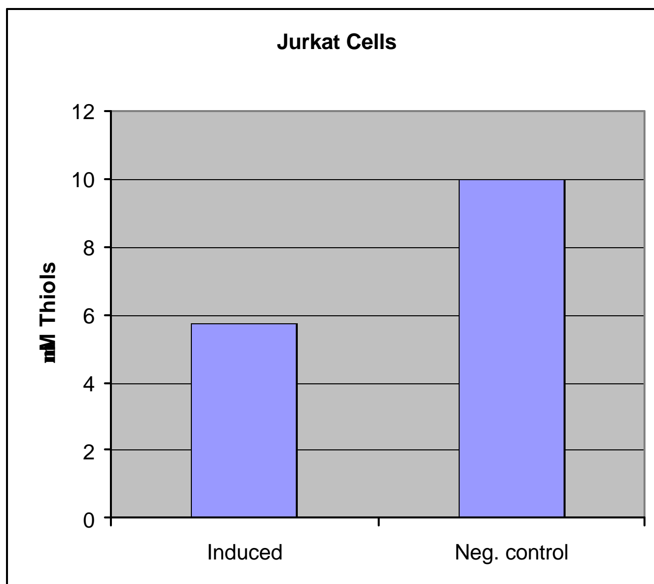

Figure 2. Jurkat cells were incubated with 1 µM staurosporine (4) for 3 hours. After which reduced thiol levels were quantified using Cell Technology's Fluoro Thiol kit. The graph represents approximately 1 x 10^4 cells per reaction (n=3).

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 activa-tion. *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).