CREATIVE BIOMART®
Assay Kit

Thiol Fluorometric Detection Kit

Product Information

Common Name

Thiol

Cat.No.

Kit-0816

Product Overview

Thiol Fluorometric Detection Kit provides an ultrasensitive fluorimetric assay to quantitate thiol content that exists either in a small molecule or on a protein. The proprietary non-fluorescent dye used in the kit becomes strongly fluorescent upon reacting with thiol. The kit can detect as little as 1 picomole of cysteine or GSH in a 100 µL assay volume (10 nM). The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The thiol sensor used in the kit generates a strongly fluorescent adduct upon reacting with a thiol compound. The resulted adduct has the spectral proterties almost identical to those of fluorescein. In addition, both absorption and emission spectra of the thiol adduct are pH-independent, making this assay kit highly robust. The signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

Description

The detection and measurement of free thiol (such as free cysteine, glutathione, and cysteine residues in proteins) is one of the essential tasks for investigating biological processes and events in many biological systems. There are a few reagents or assay kits available for quantitating thiol content in biological systems. All the commercial kits either lack sensitivity or have tedious protocols.

Usage

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.

Kit Components

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1. Green Dye: 1 Vial
2. Assay Buffer: 25 mL
3. GSH Standard: 1 Vial (62 ug)
4. DMSO: 1 vial (200 uL)

Preparation

1. Prepare GSH standard stock solution:
Add 200 uL of ddH2O into the GSH standard vial to make 1 mM (1 nmol/uL) stock solution.
Note: The unused GSH solution should be divided into single use aliquots and stored at -20 °C.
2. Prepare 100X Green Dye stock solution:
Add 100 uL of DMSO into the vial of Green Dye to make 100X stock solution.
Note: The unused Green Dye solution should be divided into single use aliquots, stored at -20 °C and kept from light.
3. Prepare GSH reaction mixture:
Add 50 uL of 100X Green Dye stock solution (from Step 2) into 5 mL of assay buffer, and mix well.
Note 1: This GSH assay mixture (GAM) is enough for two 96-well plates. It is unstable at room temperature, and should be used promptly within 2 hours and avoid exposure to light.
Note 2: Alternatively, one can make GSH Assay Mixture by adding 100X Green Dye stock solution with Assay Buffer proportionally.
4. Prepare serial dilutions of GSH standard (0 to 10 μM):
4.1 Add 30 μL of GSH standard stock solution (from Step 1) to 970 uL of assay buffer to generate 30 uM (30 pmol/uL) GSH standard.
Note: Diluted GSH standard solution is unstable. Use within 4 hours.
4.2 Take 200 μL of 30 uM GSH standard solution to perform 1:3 serial dilutions to get 10, 3, 1, 0.3, 0.1, 0.03, 0.01 and 0 μM serial dilutions of GSH standard.
4.3 Add GSH standards and GSH-containing or other thiol-containing test samples into a solid black 96-well microplate as shown in Tables 1 and 2.
Note: Treat cells or tissue samples as desired.
Table 1 Layout of GSH standards and test samples in a solid black 96-well microplate
BL BL TS TS …. ….
GS1 GS1 …. …. …. ….

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GS2 GS2
GS3 GS3
GS4 GS4
GS5 GS5
GS6 GS6
GS7 GS7
Note: GS= GSH Standards, BL=Blank Control, TS=Test Samples.
Table 2 Reagent composition for each well
GSH Standard Blank Control Test Sample
Serial Dilutions*: 50 μL Assay Buffer: 50 μL 50 μL
*Note: Add the serial dilutions of GSH standard from 0.01 μM to10 μM into wells from GS1 to GS7 in duplicate.

Assay Protocol

5. Run GSH assay:
5.1 Add 50 uL of GSH reaction mixture (from Step 3.1) to each well of the GSH standard, blank control, and test samples (see Step 4.3) to make the total GSH assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 μL of sample and 25 uL of GSH reaction mixture into each well.
5.2 Incubate the reaction at room temperature for 10 minutes to 1 hour, protected from light.
5.3 Monitor the fluorescence increase at Ex/Em = 490/520 nm with a fluorescence plate reader.

Analysis

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with the GSH reactions.
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.