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Product Information

Fluorometric Thiol Quantitation Kit
Green Fluorescence

Catalog Number **MAK151**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Fluorometric Thiol Quantitation Kit provides an ultrasensitive assay to quantitate the thiol content of small molecules. The proprietary thiol sensor generates a strongly fluorescent adduct (λ_{ex} = 490/λ_{em} = 520 nm) upon reacting with a thiol-containing compound. In addition, both absorption and emission spectra of the thiol adduct are pH-independent, making this assay kit highly robust. The kit can detect as little as 1 picomole of cysteine or glutathione (GSH) in a 100 µL assay volume (10 nM). The assay can be performed conveniently in either a 96 or 384 multiwell plate format.

Components

The kit is sufficient for 200 assays in 96 well plates.

Thiol Detection Reagent Catalog Number MAK151A	1 vl
Assay Buffer Catalog Number MAK151B	25 mL
GSH Standard Catalog Number MAK151C	1 vl
DMSO Catalog Number MAK151D	0.2 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Allow reagents to come to room temperature before use. Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

Thiol Detection Reagent – Reconstitute with 100 µL of DMSO to generate a 100× Thiol Detection Reagent stock solution. Mix well by pipetting, then aliquot and store at –20 °C.

GSH Standard – Reconstitute with 200 µL of water to generate a 1 mM (1 nmole/µL) GSH Standard stock solution. Mix well by pipetting, then aliquot and store at –20 °C. Use within 6 months of reconstitution.

Storage/Stability

The kit is shipped under ambient conditions and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

GSH Standards for Fluorometric Detection
Dilute 30 µL of the 1 mM Standard with 970 µL of Assay Buffer to prepare a 30 µM standard solution. Further dilute the 30 µM standard solution by 3-fold serial dilutions with Assay Buffer. Add 50 µL of the diluted standard solutions into a 96 well plate, generating 0 (blank), 0.014, 0.04, 0.12, 0.37, 1.1, 3.3, and 10 µM standards. Diluted standards are not stable and should be used within 4 hours.

Sample Preparation for 96 well Plate
Add 0–50 µL of sample to wells. Bring samples to a final volume of 50 µL with Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction for 96 well Plate
1. Set up the Master Reaction Mix according to the scheme in Table 1. The Master Reaction Mix is enough for one plate, as 50 µL of the Master Reaction Mix is required for each reaction (well).
   Note: The Master Reaction Mix is not stable and best used within 2 hours.

Table 1.
Master Reaction Mix

Reagent	Volume
100× Thiol Detection Reagent Stock Solution	50 µL
Assay Buffer	5 mL

2. Add 50 µL of the Master Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 10–60 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the fluorescence intensity at λ_{ex} = 490/ λ_{em} = 535 nm.

Assay Reaction for 384 well Plate
Run assay as written for 96 well plate. Use 0–25 µL of sample or standard and 25 µL of the Master Reaction Mix per well.

Results
Calculations
The background for the assay is the value obtained for the 0 (blank) GSH standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the appropriate GSH standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Using the corrected measurement, the concentration of thiol present in the samples may be determined from the standard curve.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorometric assays, use black plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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