ab112158

Thiol Quantification Assay Kit (Fluorometric)

Instructions for Use

For quantification of Thiol in small molecules using our proprietary fluorescence probe in physiological samples

This product is for research use only and is not intended for diagnostic use.

Version 7g Last Updated 16 April 2025

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1. Introduction

The detection and measurement of free thiol (such as free cysteine, glutathione, and cysteine residues) 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.

ab112158 provides an ultrasensitive fluorometric assay to quantitate thiol content that exists in a small molecule. The proprietary non-fluorescent dye used in the kit becomes strongly fluorescent upon reacting with thiol.

ab112158 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. This adduct has spectral properties almost identical to those of fluorescein. In addition, both absorption and emission spectra of the thiol adduct are pH-independent, making ab112158 highly robust. The signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/520 nm.

Kit Key Features

- **Broad Application**: Can be used for quantifying thiol and sulfide in a variety of biological systems (e.g., plasma, urine and cell extracts)

- **Sensitive:** Detect as low as 1 picomole of thiol.

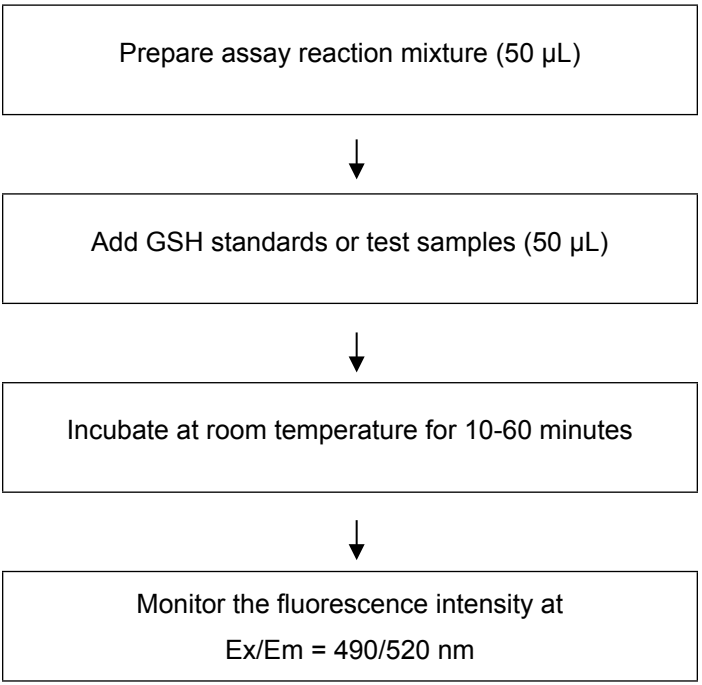
- **Continuous:** Easily adapted to automation without a separation step.

- **Convenient:** Formulated to have minimal hands-on time. No wash is required.

- **Non-Radioactive:** No special requirements for waste treatment.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: Thiol Green Indicator (light sensitive)	1 vial
Component B: Assay Buffer	25 mL
Component C: GSH Standard	62 µg
Component D: DMSO	400 µL

4. Storage and Handling

Keep at -20°C. Avoid exposure to moisture and light.

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of GSH Standard Stock Solutions

Add 200 µL of ddH_2O into the GSH standard vial (Component C) to make 1 mM (1 nmol/µL) stock solution.

Note: The unused GSH solution should be divided into single use aliquots and stored at -20 °C.

B. Preparation of 100X Thiol Green Indicator Stock Solution

Add 100 µL of DMSO (Component D) into the vial of Thiol Green Indicator (Component A) to make 100X stock solution. Please add the DMSO drop by drop, vortexing in between, to prevent formation of precipitate.

Note: The unused Thiol Green Indicator solution should be divided into single use aliquots, stored at -20 °C and kept from light.

C. Preparation of GSH Reaction Mixture

Add 50 µL of 100X Thiol Green Indicator stock solution (from Step B) into 5 mL of assay buffer (Component B), and mix well.

Note: Please add the 50 µL of 100X Thiol Green Indicator stock solution drop by drop into the 5 mL of assay buffer, vortexing in between, to prevent formation of precipitate.

D. Preparation of serial dilutions of GSH standard (0 to 30 µM)

1. Add 30 µL of GSH standard stock solution (from Step A) to 970 µL of assay buffer (Component B) to generate 30 µM (30 pmol/µL) GSH standard.

   Note: Diluted GSH standard solution is unstable. Use within 4 hours.

2. Take 200 µL of 30 µM GSH standard solution to perform 1:3 serial dilutions to get serially diluted GSH standards with Assay Buffer.

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.

BL	BL	TS	TS .....
GS1	GS1	....	....
GS2	GS2	....	....
GS3	GS3		
GS4	GS4		
GS5	GS5		
GS6	GS6		
GS7	GS7		

Table 1. Layout of GSH standards and test samples in a solid black 96-well microplate.

Note: GS= GSH Standards, BL=Blank Control, TS=Test Samples.

GSH Standard	Blank Control	Test Sample
Serial dilutions*: 50 µL	Assay Buffer: 50 µL	50 µL

Table 2. Reagent composition for each well.

**Note: Add the serially diluted GSH standards from 0.01µM to 10µM into wells from GS1 to GS7 in duplicate.*

E. Run GSH Assay:

1. Add 50 µL of GSH reaction mixture (from Step C.1) to each well of the GSH standard, blank control, and test samples (see Step D.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 µL of GSH reaction mixture into each well.

2. Incubate the reaction at room temperature for 10 minutes to 1 hour, protected from light

Monitor the fluorescence increase at Ex/Em = 490/520 nm with a fluorescence plate reader.

6. Data Analysis

The fluorescence in blank wells (with assay buffer only) is used as a control, and is subtracted from the values for those wells with GSH reactions. A GSH standard curve is shown in Figure 1.

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.

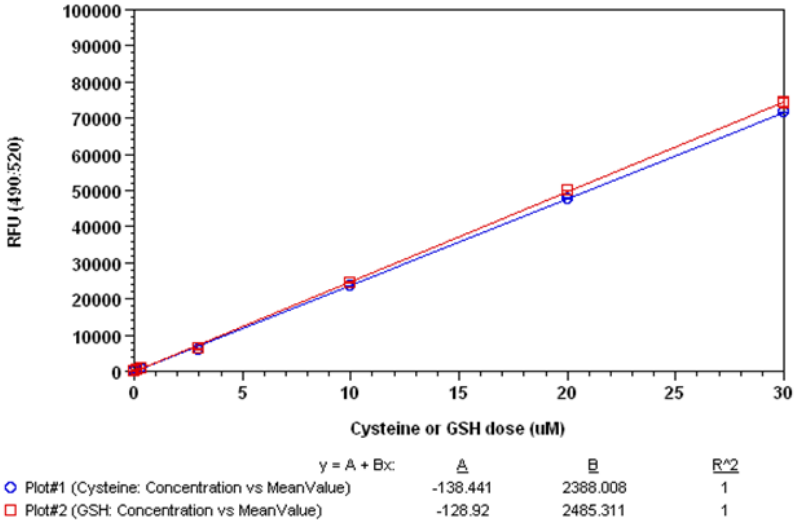


Figure 1. GSH and Cysteine dose responses were measured in a 96-well black plate with ab112158 using a microplate reader. As low as 10 nM (1 pmol/well) of GSH or Cysteine can be detected with 10 minutes incubation time (n=3).

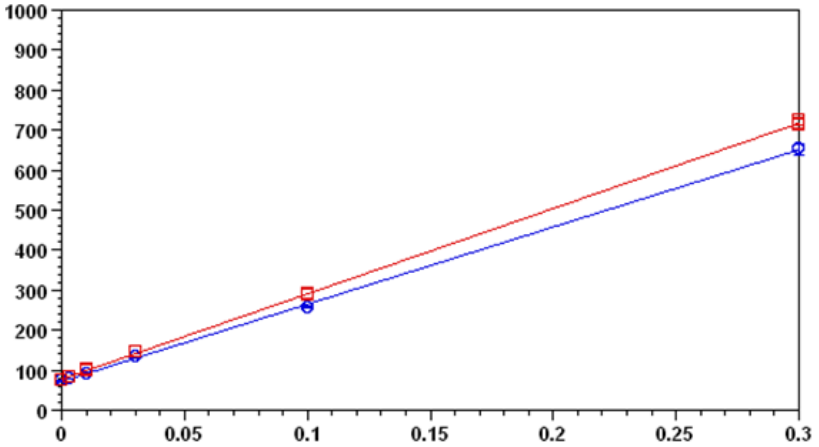


Figure 2 Shows the low levels of thiol detection possible.

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Problem	**Reason**	**Solution**
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use

	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
Lower/ Higher readings in samples and standards	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
Unexpected results	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
Standard curve is not linear	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix

	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

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