ab156049

Thiol Green Indicator

Instructions for Use

For quantifying the number of cysteines on a protein

View kit datasheet: www.abcam.com/ab156049
(use www.abcam.cn/ab156049 for China, or www.abcam.co.jp/ab156049 for Japan)

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

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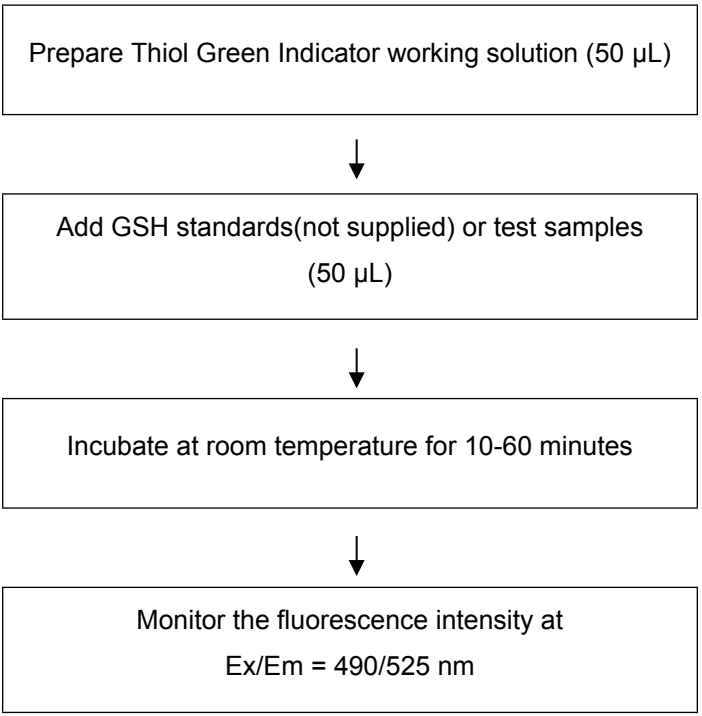
1. Introduction

ab156049 Thiol Green Indicator is one of the most sensitive sensors for measuring thiol compounds. It gives a green fluorescent adduct upon reacting with thiol compounds (such as cysteine). It can be used to quantify the number of cysteines on a protein. This product when used it to measure glutathione fluorimetrically has a >200 fold fluorescence enhancement upon reaction with thiol-containing compounds.

Spectral Properties:

Ex/Em = 510/524 nm

2. Protocol Summary



Note: This protocol is recommended for Thiol assay in solution. The protocol only provides a guideline, should be modified according to the specific needs.

3. Kit Contents

Component	Amount
Thiol Green Indicator (light sensitive)	5 mg

NOTE: Please note that this kit does not contain standards and these have to be provided by researcher.

4. Additional Materials Required

- DMSO (anhydrous)
- GSH Standard
- 20mM Hepes Buffer or buffer of choice pH7.0
- Black 96 well plate

5. Storage and Handling

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

6. Protocol

A. Preparation of Thiol Green Indicator working solution:

1. Thiol Green Indicator stock solution: Prepare a 10 to 25mM by the addition of anhydrous DMSO into the vial (mix well). The stock solution should be used promptly; any unused solution should be aliquoted and re-frozen at -20°C.

   The molecular weight of the Thiol Green Indicator is 419 Da.

   Note: Avoid repeated freeze-thaw cycles. Protect from light.

2. 2X Thiol Green Indidator Working Solution: On the day of the experiment, either dissolve HRP Substrate solid in DMSO or thaw an aliquot of the HRP Substrate stock solution at room temperature. Prepare a 2X working solution at the final concentration ranging from 100 to 250 µM in 20 mM Hepes buffer or buffer of your choice, pH 7. It is recommended to use Thiol Green Indicator at the final concentration ranging from 50 to 100 µM to measure Thiol concentration in solution.

B. Run GSH assay in supernatants:

1. Add 50 µL of 2X Thiolite™ Green working solution (from Step 1.2) to each well of the GSH standard, blank control, and test samples 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 to 60 minutes, protected from light.

3. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 490/525 nm.

4. 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 peroxidase reactions.

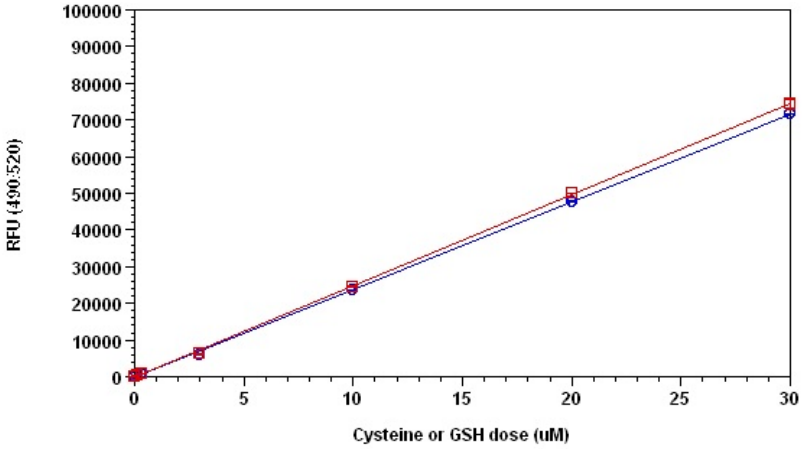


Figure 1. GSH and cysteine dose response was measured with Thiol Green Indicator on a 96-well black plate As low as 10 nM (1 pmol/well) of GSH or cysteine can be detected with 10 minutes incubation time (n=3).

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
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	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

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***.
	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
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	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)

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