

## Measure-iT™ Thiol Assay Kit (M30550)

### Quick Facts

#### Storage upon receipt:

- Store at  $\leq -20^{\circ}\text{C}$
- Protect from light
- Desiccate
- Avoid freeze/thaw cycles

**Ex/Em:** 494/517 nm

### Introduction

The Measure-iT™ Thiol Assay Kit provides easy and accurate quantitation of thiol. The kit supplies concentrated assay reagent, dilution buffer, and concentrated thiol standard. Simply dilute the reagent 1:100, load 100  $\mu\text{L}$  into the wells of a microplate, add 1–10  $\mu\text{L}$  sample volumes, mix, then read the fluorescence (Figure 1). The assay has a linear range of 0.05–5  $\mu\text{M}$  thiol, making it up to 400 times more sensitive than colorimetric methods based on Ellman's reagent. The assay is performed at room temperature; maximum fluorescence signal is attained within 5 minutes and is stable for at least 1 hour. Common contaminants are well tolerated in the assay. The kit provides sufficient material for 500 assays.

### Materials

#### Kit Contents

- **Component A:** Measure-iT™ thiol quantitation reagent, 500  $\mu\text{L}$  of a 100X concentrate in propanediol
- **Component B:** Measure-iT™ thiol quantitation buffer, 50 mL of 50 mM potassium phosphate buffer
- **Component C:** Measure-iT™ thiol quantitation standard, 30 mg of reduced glutathione

Sufficient materials are supplied for 500 assays, based on a 100  $\mu\text{L}$  assay volume in a 96-well microplate format. The Measure-iT™ thiol assay can also be adapted for use in cuvettes or 384-well microplates.

#### Storage

Upon receipt, store the kit at  $\leq -20^{\circ}\text{C}$ , desiccated and protected from light. Under these conditions the components should be stable for at least 6 months. For convenience, the Measure-iT™

thiol quantitation buffer (Component B) may be stored short term (days) at room temperature, protected from light. However, for longer periods, we recommend storage at  $\leq -20^{\circ}\text{C}$  to prevent microbial contamination.

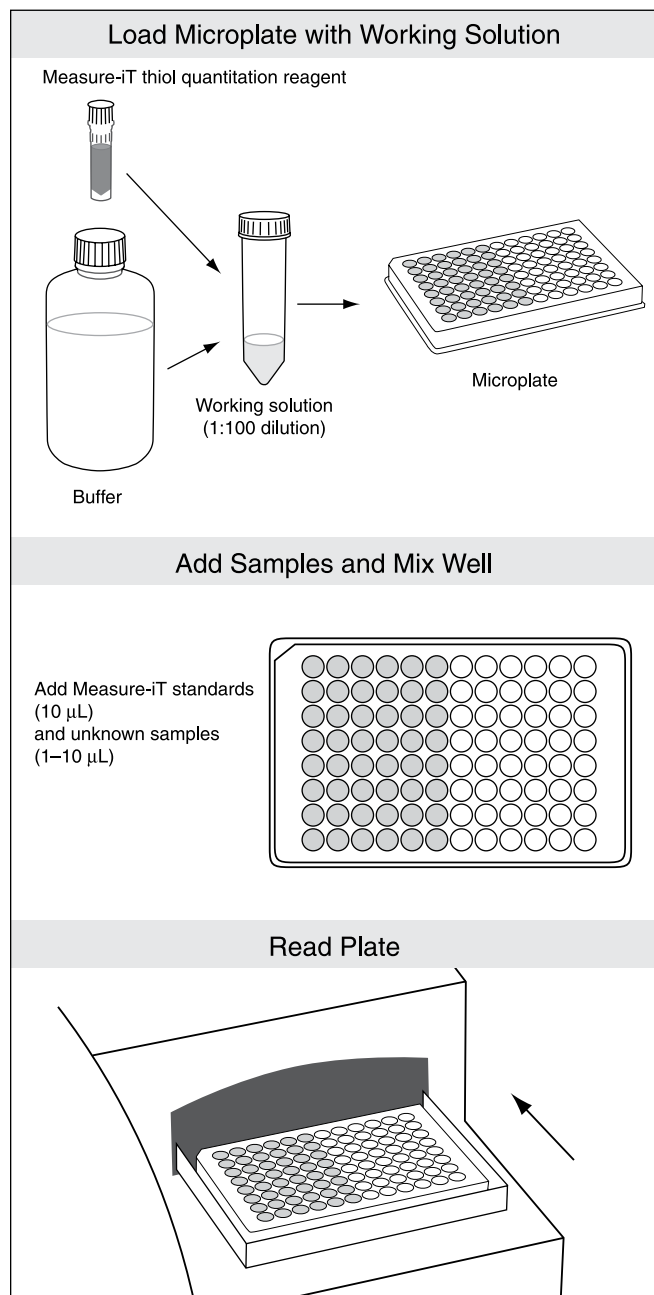


Figure 1. The Measure-iT™ Thiol Assay.

## Handling and Disposal

We must caution that no data are available addressing the toxicity of the Measure-iT™ thiol quantitation reagent. Treat the reagent with the same safety precautions as all other chemicals with unknown toxicity, and dispose in accordance with local regulations.

## Experimental Protocol

### General Considerations

During all steps, protect the Measure-iT™ thiol quantitation reagent concentrate and the working solution from light as much as possible. Traditionally, all solutions used for thiol determination are thoroughly degassed prior to use. Allow the kit components to equilibrate to room temperature before use. The assay temperature is “room temperature,” defined here as 20–25°C. Assay temperatures outside this range have not been tested but may be acceptable.

### Thiol Assay Procedure

**1. Prepare a stock solution of the Measure-iT™ thiol quantitation standard.** Dissolve 5 mg of the Measure-iT™ thiol quantitation standard (Component C) in 150 µL deionized H<sub>2</sub>O to prepare a 110 mM stock solution. This solution is stable for at least 2 months when stored at ≤6°C.

**2. Prepare a working solution of the Measure-iT™ thiol quantitation standard.** Dilute 5 µL of the 110 mM stock solution (prepared in step 1) with 4995 µL deionized H<sub>2</sub>O to prepare 5 mL of a 110 µM working solution. This solution is stable for at least 2 weeks when stored at ≤6°C.

**3. Prepare Measure-iT™ thiol quantitation standards.** Dilute the 110 µM working solution (prepared in step 2) with deionized H<sub>2</sub>O according to Table 1. Measure-iT™ thiol quantitation standards are stable for at least 1 week when stored at ≤6°C.

**4. Prepare a 1:100 working solution of the Measure-iT™ thiol quantitation reagent.** For example, for ~100 assays, dilute 100 µL of Measure-iT™ thiol quantitation reagent (Component A)

with 10 mL of Measure-iT™ thiol quantitation buffer (Component B) in a disposable plastic container and mix well. Do not use glass containers.

**5. Load 100 µL of the Measure-iT™ thiol quantitation reagent working solution prepared in step 4 into each microplate well.** For best results, diluted thiol quantitation reagent should be used within 1 hour of preparation.

**6. Add 10 µL of the Measure-iT™ thiol quantitation standards prepared in Step 3 to separate wells and mix well.** Duplicates or triplicates of the standards are recommended.

**7. Add 1–10 µL of each unknown thiol sample to separate wells and mix well.** Duplicates or triplicates of the unknown samples are recommended. Some contaminating substances may interfere with the assay (see below). For highest precision, the volumes of all reactions can be equalized by adding a small volume of the dilution buffer. Equalizing the volumes is especially important in cases where contaminating substances may be present.

**8. Measure the fluorescence using a microplate reader (excitation/emission maxima are 494/517 nm).** The maximum fluorescence signal is attained in 5 minutes, and is stable (±10%) for at least 1 hour. Fluorescence signal in the range of 0.5–5 µM thiol is stable (±10%) for at least 4 hours.

**9. Use a standard curve to determine the unknown thiol concentration.** For the thiol standards, plot the thiol concentration vs. fluorescence and fit a straight line to the data points.

## Protocol Details

### Generating Standard Curves and Extending the Assay Range

In this manual we have plotted standard curves as concentration of thiol (µM) vs. fluorescence. Alternatively, the x-axis can be expressed as the concentration of the added sample. Table 2 is provided to facilitate these unit conversions.

**Table 1.** Preparation of Measure-iT™ Thiol Standards.

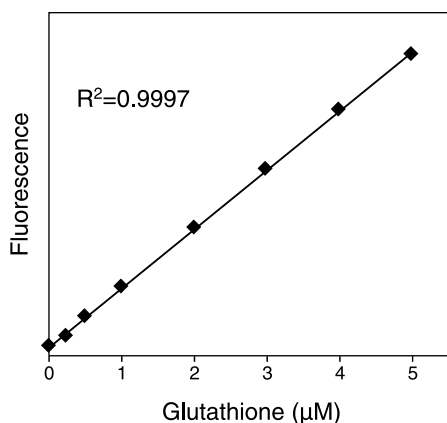
Concentration of Thiol Standard (µM)	Volume of Thiol Working Solution* (µL)	Volume of Deionized H <sub>2</sub> O (µL)
0	0	1000
2.75	25	975
5.5	50	950
11	100	900
22	200	800
33	300	700
44	400	600
55	500	500

\* 110 µM working solution of the Measure-iT™ thiol quantitation standard prepared in step 2.

**Table 2.** Concentration Conversion.\*

Final Concentration (µM)	Concentration in Given Sample Volume (µM)		
	1 µL	5 µL	10 µL
0.05	5	1	0.5
1	100	20	10
5	500	100	20

\* The Measure-iT™ thiol assay is designed to detect 0.05–5 µM thiol in a 100 µL assay volume. Sample volumes may vary from 1–10 µL; therefore, sample concentration may vary from 0.5–500 µM.

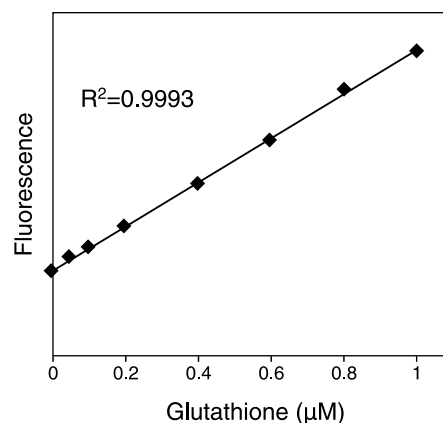


**Figure 2.** Linearity and sensitivity of the Measure-iT™ thiol assay. Triplicate 10 µL samples of glutathione were assayed; fluorescence was measured at 490/520 nm and plotted versus glutathione concentration. The variation (CV) of replicate samples was <2%.

The assay is linear from 0.05–5 µM thiol (Figure 2). For best results at the low end of the standard curve, the line should be forced through the background point (or through zero if background has been subtracted). When prepared as described above, the lowest thiol-containing standard represents 0.25 µM thiol; however, highly accurate determinations of thiol down to 0.05 µM are attained using the standard curve as described above. To assess the reliability of the assay in the lower range, use smaller volumes of the standards (e.g., 2 µL) to generate a standard curve covering the range of 0.05–1 µM (Figure 3).

### Protein Samples

Free thiol in protein samples may be quantitated with the Measure-iT™ Thiol Assay Kit. Thiol from reduced disulfide



**Figure 3.** Extended range for the Measure-iT™ thiol assay. Duplicate samples of 2 µL glutathione were assayed; fluorescence was measured at 490/520 nm and plotted versus glutathione concentration. The variation (CV) of replicate samples was <5%.

linkages in protein may also be quantitated; however, special care must be taken to remove any reducing agents from the sample media before performing the assay.

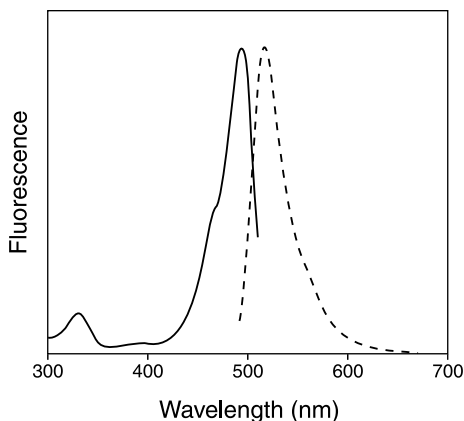
### Contaminating Substances

A number of common contaminants have been tested in the presence of thiol with the Measure-iT™ thiol quantitation assay, and most are well tolerated (Table 3). For untested contaminating substances, and for highest accuracy, the standards should be assayed under the same conditions as the unknowns. For example, if the experimental samples are in a non-standard buffer and if 10 µL volumes of these samples are used, add 10 µL volumes of the non-standard buffer (lacking thiol) when assaying the standards.

**Table 3.** Effect of Contaminants in the Measure-iT™ Thiol Assay. \*

Contaminant	Final Concentration in the Assay	Concentration in 10 µL Sample	Concentration in 5 µL Sample	Result
F(ab') <sub>2</sub>	5 µg/mL	50 µg/mL	100 µg/mL	OK
BSA	10 µg/mL	100 µg/mL	200 µg/mL	OK
IgG	5 µg/mL	50 µg/mL	100 µg/mL	OK
SDS ‡	0.1%	1%	2%	OK
Tween® 20	0.01%	0.1%	0.2%	OK
Triton® X-100	0.01%	0.1%	0.2%	OK †
Sodium azide	1 mM	10 mM	20 mM	OK
Potassium phosphate/NaCl (pH 7.2)	2 mM/3 mM	20 mM/30 mM	40 mM/60 mM	OK †
Sodium chloride	10 mM	100 mM	200 mM	OK
Magnesium chloride	10 mM	100 mM	200 mM	OK
Phenol	0.01%	0.1%	0.2%	OK
EDTA (pH 8)	10 mM	100 mM	200 mM	OK
Sodium acetate	15 mM	155 mM	300 mM	OK
Ethanol	1%	10%	20%	OK
dsDNA	5 µg/mL	50 µg/mL	100 µg/mL	OK
Glycerol	1%	10%	20%	OK
Sodium hydroxide	500 µM	5 mM	10 mM	OK

\* Glutathione standards were assayed in the presence or absence of contaminants at the indicated final concentrations. Equivalent concentrations (approximate) in 10 µL or 5 µL sample volumes are also listed. Results are given as OK = usually less than 10% perturbation. † An acceptable result, but with some distortion of the standard curve. For best results, add the same amount of contaminant to the standard samples. ‡ Precipitate formed



**Figure 4.** Normalized excitation and emission maxima for the Measure-iT™ thiol quantitation reagent bound to glutathione.

### Excitation and Emission Maxima

The excitation and emission maxima for the Measure-iT™ thiol quantitation reagent bound to glutathione are 494 and 517 nm, respectively (Figure 4). Excitation/emission settings of 490/520 nm work well in the assay.

---

## Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
M30550	Measure-iT™ Thiol Assay Kit *500 assays* .....	1 kit

---

## Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website — [probes.invitrogen.com](http://probes.invitrogen.com) — for the most up-to-date information.

### Molecular Probes, Inc.

29851 Willow Creek Road, Eugene, OR 97402  
 Phone: (541) 465-8300 • Fax: (541) 335-0504

### Customer Service: 6:00 am to 4:30 pm (Pacific Time)

Phone: (541) 335-0338 • Fax: (541) 335-0305 • [probesorder@invitrogen.com](mailto:probesorder@invitrogen.com)

### Toll-Free Ordering for USA:

Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

### Technical Service: 8:00 am to 4:00 pm (Pacific Time)

Phone: (541) 335-0353 • Toll-Free (800) 438-2209  
 Fax: (541) 335-0238 • [probetech@invitrogen.com](mailto:probetech@invitrogen.com)

### Invitrogen European Headquarters

Invitrogen, Ltd.  
 3 Fountain Drive  
 Inchinnan Business Park  
 Paisley PA4 9RF, UK  
 Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260  
 Email: [euroinfo@invitrogen.com](mailto:euroinfo@invitrogen.com)  
 Technical Services: [eurotech@invitrogen.com](mailto:eurotech@invitrogen.com)

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

### Limited Use Label License

For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300. Fax: (541) 335-0504.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2008, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.