Glutathione Fluorescent Detection Kit

Catalog Number EIAGSHF (96 tests)

Rev 1.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Gluthathione Fluorescent Detection Kit is a fluorescent detection assay designed to measure glutathione (GSH) and oxidized glutathione (GSSG) content in a variety of samples. The kit uses a proprietary non-fluorescent molecule to covalently bind the thiol group on GSH to produce a fluorescent product (390 nm excitation, 510 nm emission).

The assay measures the glutathione content in whole blood, serum, plasma, erythrocytes, urine, cell lysates, and tissue samples. The assay was characterized with human samples, but can be used to test samples from other species.

Glutathione (L-γ-glutamyl-L-cysteinylglycine) is the highest concentration non-protein thiol in mammalian cells, and is present at concentrations of 0.5–10 mM. GSH plays a key role in many biological processes, including proteins and DNA synthesis, amino acid transport, and oxidative stress protection.

Contents and storage

Kit and components are shipped at 4°C. Upon receipt, store the kit at 4°C until the expiration date on the kit box. **Do not freeze**.

Components	Quantity
Glutathione Standard; 250 µM glutathione in a special stabilizing solution	100 µL
Black 96-well Half Area Plate	1 plate
Detection Reagent; reconstitute with Dry DMSO	2 vials
Dry DMSO (Dimethyl sulfoxide)	4 mL
Assay Buffer Concentrate (2X)	35 mL
NADPH Concentrate (10X): reduced β-nicotinamide adenine dinucleotide 2'-phosphate	300 µL
Glutathione Reductase Concentrate (10X)	300 µL
Oxidized Glutathione Control	300 µL

Materials required but not supplied

- Distilled or deionized water
- Fluorescence microtiter plate reader with software capable of measurement at or near 510 nm, with excitation at 390 nm
- 37°C incubator
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Aqueous 5-sulfo-salicylic acid dihydrate (Sigma-Aldrich S2130)

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 5% SSA (w/v)

Add 1 g of aqueous 5-sulfo-salicylic acid dihydrate to 20 mL of water.

Prepare 1X Assay Buffer

1. Dilute 35 mL of Assay Buffer (2X) with 35 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. 1X Assay Buffer is stable at 2°C to 8°C for 3 months.

Prepare Sample Diluent

1. Dilute 5% SSA 1:5 with 1X Assay Buffer (e.g., add 5 mL 5% SSA to 20 mL 1X Assay Buffer) and vortex thoroughly.
2. Adjust pH of Sample Diluent to >6.
3. Store the Sample Diluent at 2°C to 8°C for 1 month.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.
- Deproteinize all samples with 5% SSA. Dilute treated samples with 1X Assay Buffer to 1% SSA.

Prepare cell lysate samples

The following procedure is used to prepare cell lysate samples. For sample preparation and dilution procedures for other sample types (whole blood, serum, plasma (EDTA and heparin), erythrocytes (RBCs), tissue, or urine), see the product page at **thermofisher.com**

Because conditions may vary, these procedures may require optimization based on sample type. After preparation, store samples on ice until assaying or freeze in aliquots for later use.

1. Wash cell pellets in ice cold PBS and resuspend in ice cold 5% SSA at 1–40 × 10^6 cells/mL.

   Note: Lysed cells in frozen samples can result in substantial amounts of GSH and GSSG in the PBS wash.
2. Lyse cells by vigorous vortexing, freeze-thaw cycling or other suitable disruption method.
3. Incubate for 10 minutes at 4°C.
4. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and collect the supernatant for analysis.

Dilute cell lysate samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application. **Use all samples within 2 hours of dilution.**

1. Dilute cell lysate samples by adding 4 volumes of Assay Buffer (the SSA concentration will be 1%, with a sample dilution of 1:5).
2. Perform an additional dilution of ≥1:4 with Sample Diluent prior to the assay (for a final dilution of ≥1:20).

Prepare Oxidized Glutathione (GSSG) Control (optional)

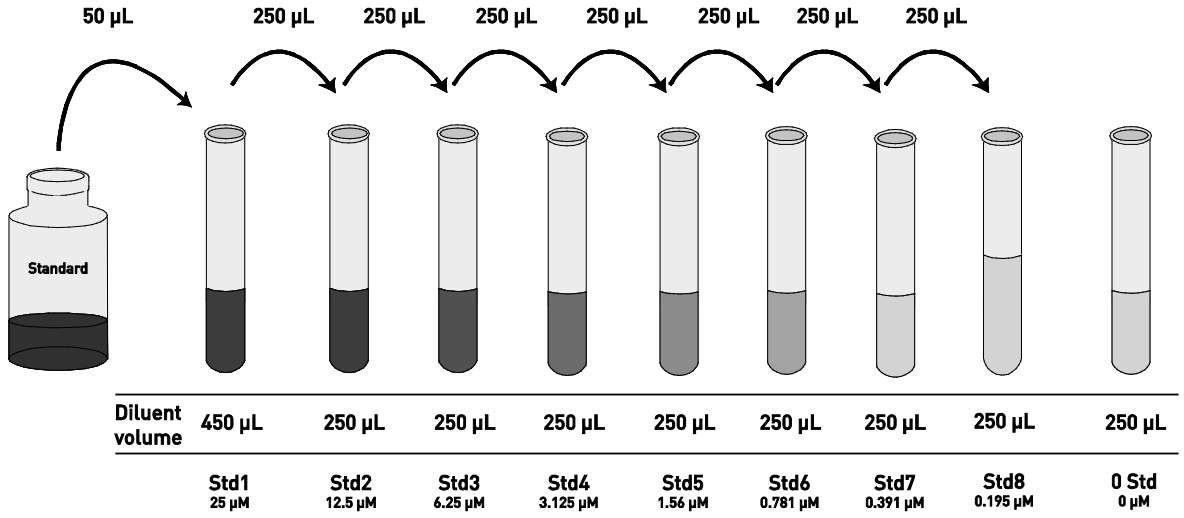
The GSSG Control solution is used to verify that the NADPH and glutathione reductase prepared in the Reaction Mixture will adequately reduce GSSG to GSH. The GSSG Control should yield a value for total GSH of approximately 10 ± 2 µM.

Add 5 µL of Oxidized Glutathione Control to 245 µL of Sample Diluent. Use within 2 hours.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Briefly centrifuge the vial of standard to ensure the contents are at the bottom of vial.
2. Add 50 µL Glutathione Standard to one tube containing 450 µL Sample Diluent and label as 25 µM glutathione.
3. Add 250 µL Sample Diluent to each of 8 tubes labeled as follows: 12.5, 6.25, 3.125, 1.56, 0.781, 0.391, 0.195, and 0 µM glutathione.
4. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
5. **Use the standards within 2 hours of preparation**.



Reconstitute Detection Reagent

Note: The Detection Reagent reacts with strong nucleophiles (e.g., buffers containing sodium azide, Proclin™, or Kathon™ preservatives).

1. Allow the Detection Reagent to reach room temperature in the sealed bag before opening.
2. Add 1.5 mL of the Dry DMSO to the vial of Detection Reagent and vortex thoroughly.

   Note: DMSO is an aprotic organic solvent shown to enhance the absorption rate of skin-permeable substances. Wear protective gloves when using the solvent, particularly when it contains dissolved chemicals.

   Store any unused reconstituted Detection Reagent at 4°C in the desiccated pouch. **Use within 2 months**.

Prepare Reaction Mixture

1. Vortex the vials of Glutathione Reductase Concentrate (10X) and NADPH Concentrate (10X).
2. Prepare Reaction Mixture according to the following table and vortex thoroughly.

Reagent	½ plate	Full plate
NADPH Concentrate (10X)	150 µL	275 µL
Glutathione Reductase Concentrate (10X)	150 µL	275 µL
1X Assay Buffer	1.2 mL	2.2 mL
Total volume	1.5 mL	2.75 mL

3. Store any unused Reaction Mixture at 4°C in an amber vial for no more than 2 days.

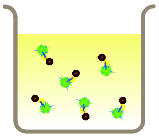
Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.

Add sample and detection reagent and measure free GSH signal

a. Add 50 µL of standards or diluted samples (see page 2) to the appropriate wells.
b. Add 25 µL Detection Reagent into each well.
c. Tap the sides of the plate and mix.
d. Incubate for 15 minutes at room temperature.
e. Read the fluorescent emission at 510 nm with excitation at 390 nm. Record the value for use in calculating free GSH concentration.



Add reaction mixture

a. Add 25 µL Reaction Mixture into each well.
b. Tap the sides of the plate and mix.
c. Incubate for 15 minutes at room temperature.

Note: To determine total GSH content only, leave out steps 1c, 1d, and 1e.

Read the plate and generate the standard curve

1. Read the fluorescent emission at 510 nm, with excitation at 390 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background fluorescence may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard and reanalyze. Multiply the concentration by the appropriate dilution factor.

Guidelines for calculating oxidized glutathione content

- Free GSH concentrations are calculated from data obtained from step 1e of the Assay procedure.
- Total GSH concentrations are calculated from data obtained from the final plate reading procedure (see Read the plate and generate the standard curve).
- The oxidized glutathione (GSSG) content is calculated by dividing the difference between the total GSH and the Free GSH by two (i.e., GSSG = ½ [total GSH – free GSH]).

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–25 µM glutathione.

Standard Glutathione (µM)	Free GSH	Total GSH
	Mean FLU	Mean FLU
25	40,945	39,976
12.5	19,737	19,034
6.25	10,006	9,958
3.125	5,269	5,814
1.56	2,671	3,429
0.781	1,571	2,209
0.391	1,009	1,816
0.195	639	1,339
0	299	1,127

Linearity of dilution

Linearity was determined by taking human RBCs at two different concentrations and mixing in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Sample %	Low Sample %	Observed Conc. (µM)		Expected Conc. (µM)		% Recovery	
		Free	Total	Free	Total	Free	Total
80	20	6.28	18.35	6.32	18.29	99.3	100.3
60	40	4.77	13.89	4.93	14.35	96.7	96.8
40	60	3.38	10.18	3.55	10.41	95.3	97.8
20	80	2.04	6.55	2.16	6.47	94.5	101.2
				Mean Recovery		96.5%	99.0%

Intra-assay precision

Samples were assayed in replicates of 20 to determine precision within an assay.

Sample	Free GSH		Total GSH	
	Mean (µM)	%CV	Mean (µM)	%CV
1	1.27	4.0	2.30	4.7
2	2.00	3.1	3.80	4.7
3	8.33	4.6	9.77	2.7
4	3.89	3.0	4.45	2.3

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 20 times in duplicate by two operators to determine precision between assays.

Sample	Free GSH		Total GSH	
	Mean (µM)	%CV	Mean (µM)	%CV
1	1.30	8.6	2.40	8.3
2	1.83	14.7	3.57	10.0
3	9.38	6.0	11.67	6.0
4	4.89	7.2	5.89	8.0

CV = Coefficient of Variation

Sensitivity

The minimum detectable dose of glutathione is 45 mM in the Free GSH and 48 nM in the Total GSH assays. This was determined by adding two standard deviations to the mean FLU obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Catalog Number

Batch code


Temperature limitation


Use by


Manufacturer


Consult instructions for use


Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

The information in this guide is subject to change without notice.

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate entity: Life Technologies Corporation | Carlsbad, CA 92008 USA | Toll Free in USA 1 800 955 6288