Glutathione Reductase Fluorescent Activity Kit

Catalog Number EIAGRF (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 Glutathione Reductase Fluorescent Activity Kit is a fluorescent activity assay designed to directly measure the activity of glutathione reductase (GR) in a variety of samples. The kit uses a proprietary non-fluorescent molecule to covalently bind the thiol product of the reaction between the substrate (GSSG) and glutathione reductase to produce a fluorescent product (390 nm excitation, 510 nm emission). The assay can be run as an end point assay, or as a kinetic activity assay.

This assay measures the activity of glutathione reductase in serum, plasma (EDTA and heparin), erythrocytes, and cell lysates. The assay was validated with human glutathione reductase, but is expected to measure glutathione reductase activity in samples from other species.

GR is a ubiquitous 100–120 kDa dimeric flavoprotein that catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione. It is involved in the prevention of oxidative damage within the cell.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity
Glutathione Reductase Standard; 200 U/mL glutathione reductase in a special stabilizing solution	40 µL
Assay Buffer Concentrate (2X)	60 mL
Black 96-well Half Area Plate	1 plate
Detection Reagent; reconstitute with Dry DMSO	1 vial
Dry DMSO (dimethyl sulfoxide)	2 mL
NADPH; reduced ß-nicotinamide adenine dinucleotide 2'-phosphate freeze dried with stabilizers stored in a desiccator	1 vial
NADPH Diluent; phosphate buffer containing detergents and stabilizers	5 mL
Oxidized Glutathione; oxidized glutathione (GSSG) in special stabilizing solution	3 mL

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 510 nm, with excitation at 390 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution

Procedural guidelines

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

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.

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.

Prepare samples

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

Sample type	Procedure
Erythrocytes (RBCs)	1. Collect blood in the presence of heparin or EDTA. 2. Centrifuge the sample and remove the plasma and white cell layer from the erythrocyte (RBC) layer. 3. Suspend the RBCs and gently wash twice with three volumes of isotonic saline (0.9%). Separate the cells by centrifugation at 600 × *g* for 10 minutes and discard the saline after each step. 4. Add four volumes of cold deionized water to the sample and vortex. 5. Incubate sample for 10 minutes at 4°C, or perform a freeze-thaw. 6. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and collect the supernatant.
Cell lysates	1. Wash cell pellets and resuspend in 1X Assay Buffer at 1–40 × 10^6 cells/mL. 2. Lyse cells by vigorous vortexing, freeze-thaw cycling or other suitable disruption method. 3. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and collect the supernatant for analysis.

Dilute samples

Sample activity should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

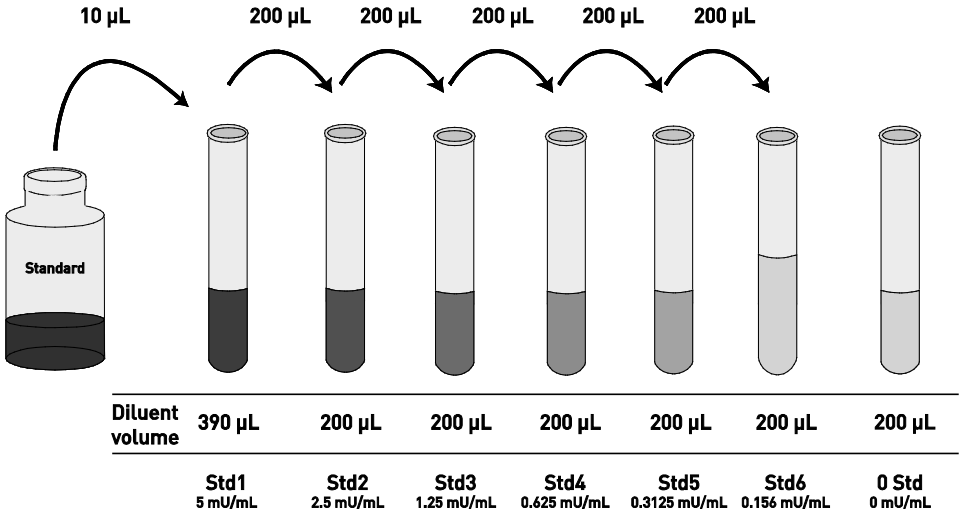
Use all samples within **2 hours** of dilution, or store at on ice until ready to perform assay.

- Dilute **serum and plasma** samples ≥1:40 in 1X Assay Buffer.
- Dilute **erythrocyte** lysate samples so that the hemoglobin content is ≤0.625 mg/mL when performing the assay. For normalization of results, measure hemoglobin levels using the Hemoglobin Colorimetric Detection Kit (Cat. No. EIAHGBC).

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 10 µL Glutathione Reductase Standard to one tube containing 390 µL 1X Assay Buffer and label as 5 mU/mL glutathione reductase.
3. Add 200 µL 1X Assay Buffer to each of 6 tubes labeled as follows: 2.5, 1.25, 0.625, 0.3125, 0.156, and 0 mU/mL glutathione reductase.
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 NADPH

1. Allow the vial of NADPH to reach room temperature in the sealed bag before opening.
2. Add 3 mL of the NADPH Diluent to the NADPH vial and vortex thoroughly.
3. Store any unused reconstituted NADPH at 4°C for no **more than 2 weeks**.

Reconstitute Detection Reagent

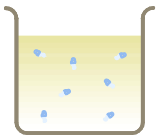
Note: The Detection Reagent reacts with strong nucleophiles (e.g., buffers containing sodium azide, Proclin™, or Kathon™ preservatives).

1. Allow the Detection Reagent to reach room temperature in the sealed bag before opening.
2. Add 1.8 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.
3. Store any unused reconstituted Detection Reagent at 4°C in the desiccated pouch. **Use within 2 months**.

Assay procedure

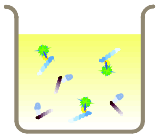
Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 20 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample and measure thiol signal

a. Add 25 µL of standards or diluted samples (see page 2) to the appropriate wells.
b. Add 15 µL of Detection Reagent to each well.
c. Tap the side of the plate to mix.
d. Incubate for 5 minutes at room temperature.
e. Read the fluorescent emission at 510 nm, with excitation at 390 nm. Record the reading for use in subtracting background thiol signal in samples.



Add substrate

a. Add 25 µL of Oxidized Glutathione into each well.
b. Add 25 µL of NADPH (see page 2) into each well.
c. Tap the side of the plate to mix.
d. Incubate for 15 minutes at room temperature.

Read the plate and generate the standard curve

1. Read the fluorescent emission at 510 nm, with excitation at 390 nm.
2. Subtract the background thiol signal readings for each well.
3. 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.
4. Read the activity of 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 in 1X Assay Buffer and reanalyze. Multiply the activity by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–5 mU/mL glutathione reductase.

Standard GR (mU/mL)	Mean FLU
5	50,450
2.5	28,768
1.25	14,429
0.625	7,609
0.3125	4,279
0.156	2,680
0	1,170

Intra-assay precision

Five samples diluted with 1X Assay Buffer were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (mU/mL)	3.35	2.38	1.75	0.56	0.27
%CV	2.7	5.6	3.9	3.7	3.6

CV = Coefficient of Variation

Inter-assay precision

Five samples diluted with 1X Assay Buffer were assayed 22 times in duplicate by four operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Mean (mU/mL)	3.35	2.36	1.64	0.62	0.27
%CV	5.0	12.6	6.8	10.6	10.5

CV = Coefficient of Variation

Performance characteristics, continued

Expected values

Ten random human serum and EDTA plasma samples were tested with the assay.

Sample	Range (mU/mL)	Average (mU/mL)
Serum/plasma	24.1–33.6	28.4

Interferents

A variety of solvents and detergents were tested as possible interfering substances in the assay.

- 5% methanol, DMSO, or DMF in the sample resulted in a <10% change in GR activity.
- 1% Tween™ 20 or Triton™ X-100 in the sample resulted in a modest increase in activity, whereas 0.01% SDS resulted in a < 3.1% decrease in activity.
- Hemoglobin levels of 0.0625% (0.625 mg/mL) in the sample resulted in a < 10% decrease in GR activity.

Linearity of dilution

Linearity was determined using lysates from high and low concentration Jurkat cell samples (high sample 2×10^5 cells/mL; low sample 2×10^4 cells/mL) mixed in the ratios shown in the following table.

Low Sample %	High Sample %	Expected Conc. (mU/mL)	Observed Conc. (mU/mL)	% Recovery
80	20	1.000	0.930	93.0
60	40	1.665	1.624	97.5
40	60	2.330	2.270	97.4
20	80	2.995	2.933	97.9
			Mean Recovery	**96.5%**

Sensitivity

The analytical sensitivity of the assay is 0.009 mU/mL glutathione reductase. 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

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REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

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