Accelerating Scientific Discovery

Elite™ Rapid Glutathione GSH/GSSG Ratio Assay Kit

Catalog Number: **CA-GS200** | Size: 2**00 assays**

1. Description

Glutathione (GSH) is the major intracellular thiol responsible for maintaining redox homeostasis. Under oxidative stress, oxidized glutathione (GSSG) accumulates, reducing the GSH/GSSG ratio. Monitoring this ratio is essential for evaluating cellular redox status, detoxification capacity, and susceptibility to oxidative injury.

The Elite™ Rapid Fluorimetric GSH/GSSG Ratio Assay Kit provides an ultrasensitive, one-step fluorimetric method for quantifying GSH and GSSG in biological samples. The assay uses a proprietary water-soluble non-fluorescent dye that becomes strongly fluorescent upon reacting with thiols. As little as **1 picomole** of GSH can be detected in a **100µL** assay volume.

The assay is compatible with **96-well and 384-well microplates**, requires no wash steps, and is easily automated.

2. Kit Components

Component	Description	Quantity / Format
Component A	Elite™ Green 520WS	1 vial (lyophilized)
Component B	Assay Buffer	1 bottle (25mL)
Component C	GSH Standard	1 vial (62µg)
Component D	GSSG Probe	1 bottle (lyophilized)
Component E	GSSG Standard	1 vial (124µg)

3. Storage

Keep in freezer (−20 °C) and avoid exposure to light. All reagents are stable for at least 6 months after receipt when stored properly at the recommended conditions.

4. Materials Required but Not Supplied

- 96-well solid black microplates with black bottom (or 384-well equivalent)
- Fluorescence microplate reader capable of E_x/E_m = 490/525 nm, cutoff = 515 nm

5. Assay Protocol (for One 96-Well Plate)

Add 50µL GSh working solution ▶ Add 50µL GSH standards, GSSG standards or test samples to each well ▶ Incubate at RT for 10–60 min ▶ Monitor fluorescence at E_x/E_m = 490/525 nm

Step 1. Preparation of Stock Solutions

1.1. **GSH Standard (1mM):** add 200µL Assay Buffer to Component C.

1.2. **GSSG Standard (1mM):** add 200µL ddH_2O to Component E.

1.3. **Elite™ Green 520WS Stock (100x):** add 100µL ddH_2O to Component A (protect from light).

Step 2. Preparation of Standard Solutions

2.1. **GSH Standards (0-10µM):** add 10µL of 1mM GSH tp 990µL Assay Buffer → 10µM (GSH7), then perform 1:2 serial dilutions to generate GSH6 → GSH1

2.2. **GSSG Standards (0-5µM):** add 10µL of 1mM GSSG tp 990µL Assay Buffer → 10µM (GSSG7), then perform 1:2 serial dilutions to generate GSSG6 → GSSG1

Note: diluted standards are unstable. Use within 4 hours.

Step 3. Preparation of Working Solutions

3.1. **GSH Working Solution (GSH-WS):** add 100µL of 100x **Elite™ Green 520WS Stock** to 10mL Assay Buffer.

Note: stable for 4 hours at 4oC, protect from light.

3.2. **Total GSH Working Solution (TGSH-WS):** add 5mL GSH-WS to Component D (GSSG Probe). Use within 2 hours.

Step 4. Sample Experimental Layout

Add the serial dilutions and test samples into a solid black 96-well microplate according to Tables 1 & 2.

Table 1. Suggested Plate Layout

BL	BL	TS	TS	TS	TS	BL	BL	TS	TS	TS	TS
GSH1	GSH1					GSSG1	GSSG1				
GSH2	GSH2					GSSG2	GSSG2				
GSH3	GSH3					GSSG3	GSSG3				
GSH4	GSH4					GSSG4	GSSG4				
GSH5	GSH5					GSSG5	GSSG5				
GSH6	GSH6					GSSG6	GSSG6				
GSH7	GSH7					GSSG7	GSSG7				

BL = Blank Control; GSH = GSH Standard; GSSG = GSSG Standard; TS = Test Sample

Table 2. Reagent Addition Guide

Well Type	Reagent	Volume
GSH Standards (GSH1–GSH7)	Serial dilutions (0.15-10μM)	50 μL
GSSG Standards (GSSG1-GSSG7)	Serial dilutions (0.078-5μM)	
Blank Control (BL)	Assay buffer	50 μL
Test Sample (TS)	Sample	50 μL

Step 5. Run the GSH/GSSG Assay

5.1. Add standards, blanks, and samples according to the table layout.

5.2. Add 50μL GSH-WS to each GSH well (total 100μL)

5.3. For Total GSH: add 50μL TGSH-WS to GSSG wells.

5.4. Incubate 10-60 minutes, protect from light.

5.5. Read fluorescence at Ex/Em = 490/525nm.

6. Data Analysis

The fluorescence intensity measured in the blank wells (assay buffer only) is used as the background control. Subtract the blank-well fluorescence values from those of all GSH&GSSG standards and test-sample wells. Plot the corrected fluorescence of the GSH&GSSG standards to generate a standard curve, and use it to determine the GSH/GSSG concentrations of the test samples (see Figure 1).

Note: Fluorescence background may increase over time. Subtract the fluorescence value of the blank wells for each time point.

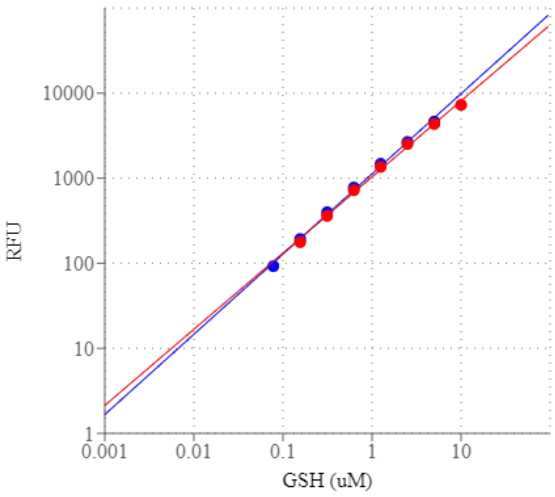

Figure1. GSH and GSSG dose responses was measured with Elite™ Glutathione GSH/GSSG Ratio Assay Kit.

Blue line: GSH dose responses (0.078 to 5µM); Red line: GSSG dose responses (0.078 to 5µM GSSG which is equivalent to 0.0156 to 10µM Total GSH).

Appendix A — GSH or GSH/GSSG Ratio Determination

The change of fluorescence intensity with GSH concentration can be described as a linear regression: Log(y) = A + B x Log(x) Note: The equation is generated by most instrument software. GSH can be calculated by the equation from the GSH standard calibration curve. Total GSH can be calculated by the equation from the Total GSH standard calibration curve.

GSSG Concentration: [GSSG] = ([Total GSH] - [GSH])/2

GSH/GSSG Ratio Determination: [GSH]/[GSSG]

Note: 0.078 to 5µM GSSG which is equivalent to 0.156 to 10µM GSH.