Sample preparation for Glutathione Colorimetric Detection Kit

Rev 1.0

Follow these instructions to prepare and dilute samples for use with the Glutathione Colorimetric Detection Kit (Cat. No. EIAGSHC).

Required materials

- Aqueous 5-sulfo-salicylic acid dihydrate (Sigma-Aldrich S2130)
- 2-vinylpyridine (Sigma-Aldrich 132292)
- Ethanol

Prepare 5% SSA (w/v)

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

Prepare Sample Diluent

1. Dilute 5% SSA 1:5 with Assay Buffer (e.g., add 5 mL 5% SSA to 20 mL 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.

(Optional) Prepare 2-vinylpyridine (2VP) solution

2-vinylpyridine (2VP) is used to block free GSH or other thiols present in samples to determine oxidized glutathione content.
Important: 2-vinylpyridine is toxic and can cause burns. Prepare solution in a fume hood.

1. Add 27 µL of 2-vinylpyridine to 98 µL of ethanol.
2. Use immediately and discard remaining unused solutions by mixing with copious amounts of water.

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 samples

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. Treat samples with 5% SSA as described in the following table:

Sample type	Procedure
Whole blood, serum, plasma (EDTA and heparin), or urine	1. Add 1 volume of cold 5% SSA to the sample and mix thoroughly, then incubate for 10 minutes at 4°C. 2. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C, and collect the supernatant. 3. (*Optional*) Centrifuge sample for 15 minutes at 4°C to clarify sample if particulate material is observed in the supernatant. 4. Collect the supernatant for analysis or store at ≤–70°C for later use.
Tissue samples requiring protein determination	1. Wash fresh tissue with ice cold PBS [1] to remove blood. 2. Homogenize every 10 mg of sample in 250 µL ice cold 100 mM phosphate buffer, pH 7. 3. Centrifuge at 14,000 rpm for 10 minutes at 4°C and remove an aliquot of the supernatant for protein determination. 4. Add 1 volume of cold 5% SSA to the remaining sample and mix thoroughly. Incubate for 10 minutes at 4°C. 5. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and collect the supernatant for analysis.
Tissue samples not requiring protein determination	1. Wash fresh tissue with ice cold PBS [1] to remove blood. 2. Homogenize every 10 mg of sample in 250 µL ice cold 5% SSA, then incubate for 10 minutes at 4°C. 3. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and collect the supernatant for analysis.
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 250 µL of RBCs to 1 mL of ice cold 5% SSA, then incubate for 10 minutes at 4°C. 4. Centrifuge samples at 14,000 rpm for 10 minutes at 4°C and collect the supernatant.
Cell lysates	1. Wash cell pellets in ice cold PBS [1] and resuspend in ice cold 5% SSA at 1–40 × 10^6 cells/mL. 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.

[1] Lysed cells in frozen samples can result in substantial amounts of GSH and GSSG in the PBS wash.

2. Proceed to "Dilute samples" if measuring **total GSH content**.
3. (*Optional*) Treat samples with 2VP if measuring **GSSG content**.
   a. Add 5 µL of 2VP solution for every 250 µL of sample, and incubate for 1 hour at room temperature.
   b. Proceed to "Dilute samples".

Note: **Free GSH content** is calculated from the difference between the **total GSH content** and **GSSH content**.

Dilute 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.**

Sample type	Procedure
Whole blood, serum, plasma (EDTA and heparin), or urine	1. Dilute samples by adding 1.5 volumes of 1X Assay Buffer (the SSA concentration will be 1%, and the sample will have been diluted 1:5). 2. Perform additional dilution with Sample Diluent. • Dilute **treated whole blood** samples at least 1:20 with Sample Diluent (final dilution ≥1:100). • Dilute **treated plasma (EDTA and heparin) or urine** samples with 1X Assay Buffer as needed.
Tissue samples requiring protein determination	1. Dilute samples by adding 1.5 volumes of 1X Assay Buffer (the SSA concentration will be 1%). 2. Perform any additional dilutions with Sample Diluent.
Tissue samples not requiring protein determination	1. Dilute samples by adding 4 volumes of 1X Assay Buffer (the SSA concentration will be 1%). 2. Perform any additional dilutions with Sample Diluent.
Erythrocytes (RBCs)	1. Dilute samples by adding 3 volumes of 1X Assay Buffer (the SSA concentration will be 1%, and the sample will have been diluted 1:20). 2. Perform any additional dilutions with Sample Diluent (human RBCs must be diluted 1:100–1:200 to be read within the standard curve).
Cell lysates	1. Dilute samples by adding 4 volumes of 1X Assay Buffer (the SSA concentration will be 1%, and the sample will have been diluted 1:5). 2. Dilute samples ≥1:20 with Sample Diluent.

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