Sample preparation for Glutathione Fluorescent Detection Kit

Rev 1.0

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

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 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 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.

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.

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 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 at least ≥1:4 with Sample Diluent (final dilution ≥1:20).

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Product label explanation of symbols and warnings

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