GSH:GSSG ANALYSIS

The Redox Molecular Signaling Core utilize Shimadzu Prominence 20 series HPLC to quantify reduced and oxidized glutathione (GSH, GSSG) in the plasma, cell, or tissue samples. 1-fluoro-2,4-dinitrofluorobenzene (dinitrofluorobenzene, DNFB or FDNB) reacts with GSH and GSSG, which can be quantified by HPLC with using UV detectors. The standards curve range of GSH and GSSG are 50~1500 μM and 2-500 μM, respectively. To initiate GSH/GSSG Measurements, contact the Redox Molecular Signaling core facility at **RedoxMolSignalCore@lsuhsc.edu** and schedule a meeting with Core Leaders to discuss the project timeline and deliverables.

To be provided by investigator:

- A completed Work Order Form brought to the meeting with Core Leaders.
- Schedule sample processing date with core due to the poor stability of the labelled GSH/GSSG samples.
- The control and unknown samples which are prepared according the following SOP (prepared in the dark due to light sensitive components).

To be generated by the core:

- GSH and GSSG HPLC analysis report

Timeline: Report will be received within 5 days.

Equipment and materials:

- DNFB, 1-Fluoro-2,4-dinitrobenzene (Sigma-Aldrich, cat. No.D1529)
- GSH, Glutathione ((Sigma-Aldrich, cat. no. G4251)
- GSSG, (Sigma-Aldrich, cat. no. G-6654)
- NEM (Sigma-Aldrich, cat. no. E1271)
- Dichloromethane (DCM; Sigma-Aldrich, cat. no. 676853)
- Trichloroacetic acid (TCA; Sigma-Aldrich, cat. no. T6399)
- Centrifuge

Procedure:

- Use cell or tissue lysis buffer (RIPA buffer) containing 100 mM NEM (200-300 μl per well of a 6-well plate). Centrifuge lysates for 8 minutes at 12,000g at 4°C.
- Transfer 200 μl of lysates into new microcentrifuge tubes. Keep samples on ice.

- PI will also submit a blank sample (triplicate, lysis buffer alone) and a control sample (triplicate) with known GSH (0.5 mM) and GSSG (200 μM) concentrations for quality control.
- Add 200 μl of10% TCA and incubate for 30 min.
- Centrifuge 12000 rpm for 10 min.
- Transfer 350 μl of supernatant into a new tube, then add 1.7 ml of DCM.
- Shake vigorously for 5 min;
- Centrifuge 8000 rpm for 5 min
- Transfer 200 μl of supernatant into new tubes filled with 100 μl of 1 M Tris-HCl (pH 10.0)
- Adjust pH to 7.5~8.0 by 6 M HCl. Specialized pH meter for this step is currently available in the BRI Rm. F6-12. This equipment will be housed in the Medical School Rm. 3-449 following core renovations.
- Add 300 μl of DNFB (1.5%, dissolved in ethanol), and incubate them at room temperature in the dark for 3 h.
- Add 50 μl of 37% HCl
- Centrifuge and storage the supernatant at 4°C. Sample must be processed by the Core Facility within 24 hours due to poor sample stability.