SULFIDE ANALYSIS

The Redox Molecular Signaling Core utilizes a Shimadzu Prominence 20 series HPLC to quantify free sulfide and sulfide pool (acid-labile sulfide and bound sulfane sulfur) in the plasma, cell, or tissue samples. A fluorescent reagent monobromobimane (MBB) reacts with free sulfide, forming sulfide-dibimane which can be quantified by HPLC with using fluorescence detectors. The limit of detection for sulfide-dibimane is 2 nM and the SDB product is very stable over time, allowing batch storage and analysis. To initiate sulfide bioavailability 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. Indicate whether free sulfide or sulfide pool measurements are requested.
- The test samples prepared according the following procedures for cell and tissue lysis.

To be generated by the core:

- Sulfide HPLC analysis report

Timeline: Timeline may depend on sample number and core schedule. Typical timelines range around 2 weeks.

Equipment and materials

- Tris-HCl
- Triton X-100
- Diethylenetriaminepentaacetic acid (Sigma D6518)
    - Prepare a 50 ml solution of 100 mM stock in ddH2O. Add 1.8 ml of 1M NaOH and place onto a rocker until the DTPA is in solution. Ensure that no crystals remain in the solution.
- Microcentrifuge tubes
- Cryovials
- Liquid nitrogen
- N_2 gas

Cell samples: Prepare chilled and degassed lysis buffer (100 mM Tris-HCl, pH 9.5, 0.1 mM DTPA, 0.1% Triton X-100). Degassing can be performed by bubbling with N_2 gas for at least 15 minutes or by placing in the hypoxia chamber overnight. Rinse cells quickly with ice cold PBS, and lyse cells using the prepared lysis buffer (approximately 200 μl per confluent well of a 6 well plate). Ensure complete lysis by passing the mixture through a p200 pipette tip ten times or by sonication (2 X 10 sec.) at 20 KHz. Centrifuge samples at 12,000 g for 5 minutes. Collect the supernatants in a fresh cryovial. Keep 20 μl for determining protein concentration within the investigators lab. Blow N_2 gas into the headspace of the cryovials, and snap-freeze the remaining 180 μl in liquid nitrogen. To safeguard the quality of the samples, ensure that no longer than 15 minutes pass between cell lysis and freezing the samples. Transfer cryovials to the liquid nitrogen dewer in 3-449 for analysis in the core. If not transferring to the core facilities immediately, store in liquid nitrogen until further analysis. Samples should be transferred to the core for derivatization and analysis within 1 week. Provide a cryovial containing lysis buffer alone for background level measurements.

Tissue samples: For tissue samples, tissue should undergo a saline perfusion to deplete tissue of red blood cells, and the time between euthanasia and freezing the samples should be kept to a minimum. Tissue should be weighed and placed into a microcentrifuge tube with chilled and degassed stabilization buffer (100 mM Tris-HCl, pH 9.5, 0.1 mM DTPA) at 10 μl per mg of tissue. Degassing can be performed by bubbling with N_2 gas for at least 15 minutes or by placing in the hypoxia chamber overnight. Homogenize the tissue quickly (within 10 minutes), and centrifuge the samples for 5 minutes at 12,000g at 4°C. Take $1/10^{th}$ of the supernatant for protein determination in the investigator's laboratory, and transfer the remaining supernatant to cryovials. The core facility requires 30 μl of sample for free sulfide measurements and 120 μl of sample to assess the sulfide pools. Blow N_2 gas into the headspace of the cryovials, and snap-freeze the cryovials in liquid nitrogen. Transfer cryovials to the liquid nitrogen dewer in 3-449 for analysis in the core. If not transferring to the core facilities immediately, store in liquid nitrogen until further analysis. Samples should be transferred to the core for derivatization and analysis within 1 week. Provide a cryovial containing lysis buffer alone for background level measurements.

Blood samples: Avoid glass for blood collection, as it will significantly bind sulfide in the samples. For blood samples, collect the blood in plastic vacutainer tubes containing lithium heparin and spin at 1500 RCF for 4 minutes at 4°C. Plasma should be immediately transferred into cryovials. Blow N_2 gas into the headspace of the cryovials, and snap-freeze the cryovials in liquid nitrogen (should be done within 15 minutes of euthanasia). The core facility requires 30 μl of sample for free sulfide measurements and 120 μl of sample to assess the sulfide pools. Collect 5 μl of red blood cells and lyse cells with 15 μl of RBC lysis buffer (10 mM Tris HCL, pH 7.5, 155 mM NH_4Cl). Dilute the red cell fraction in cryovials containing 380 μl degassed stabilization buffer (100 mM Tris-HCl, pH 9.5, 0.1 mM DTPA). Blow N_2 gas into the headspace of the cryovials, and snap-freeze the cryovials in liquid nitrogen. Transfer cryovials to the liquid nitrogen dewer in 3-449 for analysis in the core. If not transferring to the core facilities immediately, store in liquid nitrogen until further analysis. Samples should be transferred to the core for derivatization and analysis within 1 week. Provide a cryovial containing lysis buffer alone for background level measurements.