REACTIVE NITROGEN SPECIES MEASUREMENTS

The Redox Molecular Signaling Core utilize Sievers Nitric Oxide Analyzer (NOA) 280i to quantify reactive nitrogen species in plasma, cell, or tissue samples. The Sievers NOA can detect NOx including nitrate, nitrite, and nitrosothiols. To initiate nitric oxide 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, indicating whether nitrate, nitrite or nitrothiols are to be measured, brought to the meeting with Core Leaders.
- The control and unknown samples which are prepared according the following SOP.

To be generated by the core:

- Nitric oxide analyzer report

Timeline: Timeline may depend on sample number and core schedule. Typical timelines range around 1 week.

Equipment and materials:

- Potassium ferricyanide (Sigma-Aldrich, cat. No. 702587)
- NEM, N-ethylmaleimide (Sigma-Aldrich, cat. no. E1271)
- Nonidet P-40 (DCM; Sigma-Aldrich, cat. no.)

Procedure:

- Prepare the NOx stabilization buffer containing 800 mM potassium ferricyanide, 17.6 mMN-ethylmaleimide, and 6% Nonidet P-40.
- Mix 100 µl of stabilization buffer and 100 µl of the biological sample lysate or liquid sample. RIPA buffer should be used to lyse cells and tissues. Liquid samples can be directly mixed with stabilization buffer. Protein quantification in the sample should be performed prior to mixing with stabilization buffer if the results are to be normalized to protein levels.
- Immediately freeze samples with liquid nitrogen and store in liquid nitrogen until transfer to the Analytical Redox Biology Sub-Core. Samples are stable in liquid nitrogen for a few months.