

SOP for whole blood collection and processing from experimental small animals (e.g., mice) for NMR metabolomics

Supplies/materials needed:

- Blood collection (e.g., needles, syringes) per ULAM technique guidelines
- Cryo-screw top tubes (1.5mL) – sterile (or autoclaved)
- Sodium porcine heparin- sterile, pharmaceutical grade – 1000 USP Units/mL; record manufacturer, lot number and expiration date of heparin product. Also, reserve a small volume (~100-200 μ L)- label and freeze (-20C).
- Ice/water bath

In planning the experiment, it is critical to consider the time of day of sample collection. One of the most important variables is the consistency of the time of day of sample collection. If samples are collected from different treatment groups on different days, care should be taken to collect corresponding samples at the same time of day. The date and TIME samples are collected should be recorded.

The minimum whole blood sample volume for NMR metabolomics is 500 μ L; a volume of 1mL is ideal. Blood samples should be obtained in accordance with Unit for Laboratory Animal Medicine (ULAM) guidelines, however, tail-vein nicking is not recommended since this procedure may result in the release of vasoactive substances that may influence the metabolome. See the ULAM guidance for details about blood collection procedures. Depending on the approach used, the blood sample from two or more animals may need to be pooled in order to achieve an ideal sample volume.

In advance of blood collection, cryo-screw top tubes (1.5mL) should be autoclaved if not already sterile. Prepare tubes for sample collection, by adding a volume of pharmaceutical grade unfractionated sodium (porcine) heparin that is equivalent to 15 USP units per each mL of blood (e.g., 15 μ L of 1000U/mL) to each needed cryo-tube. Tubes can be capped and kept at room temperature if sample collection is imminent or at 4°C for up to 24h.

Immediately upon collection of sample, transfer it to the prepared sample tube and completely and gently invert each tube at least five times. Samples can remain on ice after collection for no more than 15 min after which they should then be placed in the freezer (-80°C).

Processing at assay site:

1. Remove samples from dry ice upon receipt.
2. Store in -80°C freezer until the day of assay.
3. On the day of assay, thaw samples on ice
4. Extract whole blood samples for NMR per protocol