

Brenner Laboratory Blood Collection Protocol for Quantitative Targeted NAD Metabolomics^{1, 2}

Required Materials

- BD Vacutainer EDTA 10ML Blood Collection Tubes (BD 366643)
- 25 mg Immucillin H (Cayman 30475)
- 1.5 mL Sterile Microcentrifuge Tubes (ThermoFisher 3457)
- 500 mL Sterile Storage Bottle
- Dry Ice Container or Liquid Nitrogen Dewar
- Dry Ice or Liquid Nitrogen
- Deionized Water
- P1000 and P20 pipette and pipette tips
- -80° C Freezer

Creating 380 μ M and 5 μ M Immucillin H Stocks

- 1) Weigh 10 mg Immucillin H and add to a 500 mL sterile storage bottle. Add 100 mL deionized H₂O to the storage bottle to create a 380 μ M Immucillin H stock solution.
- 2) Microaliquot 14 μ L of the 380 μ M Immucillin H solution using a P20 pipette to an appropriate number of 1.5 mL sterile centrifuge tubes for storage at -20° C. Remaining 380 μ M Immucillin H can be frozen in 15 ml or 50 ml conical tubes.
- 3) To 14 μ L of the 380 μ M Immucillin H solution, add 986 μ L deionized H₂O using a P1000 pipette to create 5 μ M Immucillin H Stock. 5 μ M Immucillin H Stock solution is treated as a 50x for blood and can be further subaliquoted for -20° C storage.

Blood Collection

- 1) Prior to blood collection, label 3 sterile 1.5 mL centrifuge tubes per blood donor. Add 2 μ L of the 5 μ M Immucillin H stock to each tube and place on ice.
- 2) Collect ~1 mL blood in the BD vacutainer tubes.
- 3) Carefully transfer 100 μ L of freshly drawn blood to the 2 μ L of the 5 μ M Immucillin H stock. Triplicate 100 μ L sample collection is recommended.
- 4) Immediately place collected blood-Immucillin H samples on dry ice or in liquid nitrogen until storage in a -80° C freezer.
- 5) Ship all samples in Styrofoam boxes packed with dry ice using overnight shipping Monday-Wednesday to:

Roger Moore
Mass Spectrometry Core
Beckman Research Institute
1500 E. Duarte Road
Hilton Building 108, room H124
Duarte, CA, 91010
626-399-1536

REFERENCES

1. Trammell, S.A. & Brenner, C. Targeted, LCMS-based Metabolomics for Quantitative Measurement of NAD(+) Metabolites. *Comput Struct Biotechnol J* **4**, e201301012 (2013).
2. Trammell, S.A. *et al.* Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat Commun* **7**, 12948 (2016).