

## Blood Collection Protocol for Quantitative Targeted NAD Metabolomics

### 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
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- Deionized Water
- P1000 and P20 pipette and pipette tips
- -80° C Freezer

### Creating 380 $\mu$ M and 5 $\mu$ M Immucillin H Stocks

- 1) Weigh 10 mg Immucillin H and add to a 500 mL sterile storage bottle. Add 100 mL deionized H<sub>2</sub>O to the storage bottle to create a 380  $\mu$ M Immucillin H stock solution.
- 2) Microaliquot 14  $\mu$ L of the 380  $\mu$ 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  $\mu$ M Immucillin H can be frozen in 15 ml or 50 ml conical tubes.
- 3) To 14  $\mu$ L of the 380  $\mu$ M Immucillin H solution, add 986  $\mu$ L deionized H<sub>2</sub>O using a P1000 pipette to create 5  $\mu$ M Immucillin H Stock. 5  $\mu$ 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  $\mu$ L of the 5  $\mu$ 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  $\mu$ L of freshly drawn blood to the 2  $\mu$ L of the 5  $\mu$ M Immucillin H stock. Triplicate 100  $\mu$ 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:

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