



## Nucleus Protocols

# Make Amino Acid Mix

## 1. PROTOCOL

- **Prepare Amino Acid Stock Solutions.**
  - Use the included Amino Acid Mix worksheet included as a resource for this protocol to determine the minimum mass you will need for each amino acid given how much amino acid mix you want to make
  - Weigh Tyr into the cap of a 15 mL conical tube. Note mass and recalculate AA mix volume based on required volume to bring Tyr to 3.25 mM
  - Label 19x low bind protein tubes for each amino acid stock other than Tyr
  - Weigh each amino acid and note mass.
    - NOTE: use static gun to remove static from each tube if needed
    - weigh mass.
    - NOTE: make sure mass stabilizes before recording
    - check if resuspension volume will fit assigned tube. else, adjust mass
  - Filter ultrapure water with 0.22  $\mu$ m and add resuspension volume of filtered ultrapure water to each tube
  - resuspend 19x amino acid stocks (excluding Tyr)
    - microfuge tubes on heater shaker set to 65C / 1400 rpm
    - note incubation start time:
    - check for complete resuspension every 30 min (should take  $\leq$  1 hr)
- **Assemble Amino Acid Master Mix.**
  - Put Tyr 15 mL tubes in hot water bath sonicator set to 65C. Keep other 19x amino acid stocks on heater shaker at 65C.
  - For each amino acid stock other than Tyr, add the calculated volume to Tyr 15 mL tube. Keep the Tyr tube in 65C sonicator bath to maintain temperature and speed up resuspension.
  - Add filtered ultrapure water to final concentration as specified
  - Resuspend amino acid mix in hot water bath sonicator set to 65C with sonication
    - Note incubation start time:
    - Check for complete resuspension every 30 min (should take  $\sim$  30 min)
  - Syringe filter using 0.22  $\mu$ m filter

### 1.1. Storage

- Aliquot and store at  $-20^{\circ}\text{C}$