

Nucleus Protocols

Make Amino Acid Mix

1. Prepare Amino Acid Stock Solutions.

- ☐ Use the included Amino Acid Mix worksheet included as a resource for this protocol to calculate required masses and volumes.
- ☐ Weigh Tyr into the cap of a 15 mL conical tube. Note mass and recalculate required final volume of AA mix to bring Tyr to 3.25 mM.
- ☐ Label 19x microfuge tubes, one for each amino acid other than Tyr.
- ☐ Weigh each amino acid into a separate tube and note mass. Make sure reading stabilizes before noting.
- ☐ Check if resuspension volume will fit assigned tube. Else, adjust mass.
- ☐ Filter ultrapure water (0.22 µm) and add the corresponding calculated resuspension volume to each tube of powdered amino acids.
- ☐ Resuspend 19x amino acid stocks (excluding Tyr).
    - ☐ Put each microfuge tube on a heater shaker set to 65 °C / 1400 rpm.
    - ☐ Check for complete resuspension every 30 min (should take ≤ 1 h). Keep stocks at 65 °C for next step.

2. Assemble Amino Acid Master Mix.

- ☐ Put Tyr 15 mL tubes in hot water bath sonicator set to 65 °C. Keep other 19x amino acid stocks on heater shaker at 65 °C.
- ☐ For each amino acid stock other than Tyr, add the calculated volume to Tyr 15 mL tube. Keep the Tyr tube in 65 °C sonicator bath to maintain temperature and facilitate resuspension.
- ☐ Add filtered ultrapure water to final concentration as specified.
- ☐ Resuspend amino acid mix in hot water bath sonicator set to 65 °C with sonication.
    - ☐ Note incubation start time:
    - ☐ Check for complete resuspension every 30 min (should take ∼ 30 min).
- ☐ Syringe filter using 0.22 µm filter.

3. Storage

- ☐ Aliquot and store at –20 °C.