



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 \leq 1 hr). 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 \sim 30 min).
- Syringe filter using 0.22 μm filter.

3. STORAGE

- Aliquot and store at -20°C.