



Nucleus Protocols

Make Protein Mix

1. STEP 1: COMPUTE MIX VOLUMES

- ☐ Open the [Protein Mix Assembly Calculator](#) and follow the directions therein.
 - ☐ Enter how many 10 μ L PURE reactions you'd like to prepare next to "PURE Reactions (#)" (default: 200) into the blue-highlighted cell. This defines the total volume of PMix needed.
 - ☐ Enter the concentrations for each of your protein stocks into the blue highlighted cells under "Stock Conc (ng/ μ L)". This is needed to calculate the required volume for each protein stock.
 - ☐ Check the "Checklist" tab for any yellow-highlighted cells and resolve flagged items before proceeding.

2. STEP 2: ASSEMBLE PROTEIN MIX

- ☐ Thaw protein stocks on ice.
- ☐ Combine proteins according to the volumes calculated in Step 1.
 - ☐ Pipette each protein into a single microfuge tube in the order listed on the calculator sheet.
 - ☐ Mix gently by pipetting after all proteins have been added.

3. STEP 3: WASH AND CONCENTRATE

- ☐ Wash assembled PMix by diluting 10x in Protein Buffer (e.g., 2.4 mL Protein Buffer for 240 μ L PMix).
- ☐ Concentrate using a 3 kDa Amicon centrifugal filter until the mix is at or above your target final concentration.

4. STEP 4: MEASURE TOTAL PROTEIN CONCENTRATION

- ☐ Prepare 10x dilutions of Protein Mix in triplicate (1 μ L Protein Mix + 9 μ L ultrapure water per replicate).
- ☐ Measure total protein concentration by [Pierce660 Assay](#) on the diluted triplicates.
- ☐ Calculate total protein concentration: average the three replicates and multiply by 10.

5. STEP 5: DILUTE TO FINAL CONCENTRATION

- ☐ If concentration exceeds target, dilute PMix to 15 mg/mL in Protein Buffer (30% glycerol).
 - ☐ Add the calculated volume to reach target concentration and mix gently.
- ☐ If concentration is below target, return to Step 3 — concentrate further, then re-measure.

6. STEP 6: ALIQUOT AND STORE

- ☐ Aliquot PMix into PCR tubes or microcentrifuge tubes.
- ☐ Store aliquots at -80°C .