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 µL 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/µL)". 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 µL 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 µL Protein Mix + 9 µL 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.