Nucleus Protocols

Purify Proteins by Ni^{2+} Gravity Column

1. Prepare Columns

- ☐ **Prepare buffers:** Prepare the following buffers; chill at 4 °C:
  - ☐ 50 mL Wash Buffer per sample.
  - ☐ 6 mL Elution Buffer per sample.
- ☐ **Assemble columns:** Prepare one (1) gravity column per sample:
  - ☐ Cut the tips off the columns with scissors or a razor blade.
  - ☐ Pack a filter into the bottom of each column (we use the back end of a cell spreader).
- ☐ **Wash empty columns:** Clamp the columns to a lab stand with a large beaker (≥ 500 mL) underneath to capture flowthrough. Wash each column and filter with 10 mL Ultrapure water.
- ☐ **Load and equilibrate resin:**
  - ☐ Resuspend the Ni^{2+} resin (50% v/v slurry) by shaking.
  - ☐ Add 2 mL resin slurry (≈ 1 mL settled bed) to each column by pipette.
  - ☐ Equilibrate each column with ≥ 10 CV (10 mL) cold Wash Buffer (4 °C); discard the flowthrough.

2. Purify Protein

- ☐ **Load lysate:** Load up to 10 CV (10 mL) clarified lysate per column. Optionally capture flowthrough for later analysis.
- ☐ **Wash:** Wash each column with ≥ 10 CV (10 mL) cold Wash Buffer. Allow buffer to flow through. Optionally capture flowthrough for later analysis.
- ☐ **Elute:** Elute sample with 6 CV (6 mL) cold Elution Buffer. Capture flowthrough in a 15 mL centrifuge tube.

3. Storing Columns

- ☐ **Short-term (up to 48 h):** Seal each column with a cap and bung and store at 4 °C.
- ☐ **Long-term / reuse:** To store columns longer than 48 h — or to save used columns for later reuse — store them in 20% EtOH. Wash the column in Wash Buffer (as above), then with 10 CV (10 mL) Ultrapure water to remove salts. Fill the column with 20% EtOH, seal the bung and lid with parafilm, and store at 4 °C.