Protocol

Cell Lysis for His-tagged Protein Purification using the AmMag™ Quatro ProAb 1300

Materials

- Magnetic beads
  - AmMag™ Ni beads, Cat. No. L00776
  - Ni MagBeads, Cat. No. L00295
- Magnetic separation tools
  - AmMag™ MR-mini (Cat. No. L00722)
  - AmMag™ MR (Cat. No. L00723)
  - AmMag™ Quatro 100mL Centrifuge Tube Magnetic Rack (Cat. No. D00066)
- Buffers
  - Resuspension & lysis: 50 mM Tris pH 8, 150 mM NaCl, 2 mM $MgCl_2$, 1 mM PMSF, 25 U/mL Benz-Neburase™, tag-free (Cat. No. Z03695-10)[1]
    - Enzymatic: + 1 mg/mL lysozyme
    - Chemical: + 1X BugBuster
    - Enzymatic + Chemical: + 1 mg/mL lysozyme + 1X BugBuster
  - Binding/Wash: 50 mM Tris pH 8.0, 150 mM NaCl
  - Elution: 50 mM Tris pH 8.0, 150 mM NaCl, 500 mM imidazole

Methods

Bead Preparation

1. Completely resuspend the magnetic beads by shaking the vial.
2. Transfer the required volume of slurry into a clean tube.
3. Place the tube on a magnetic separation rack to collect the beads. Remove and discard the supernatant.
4. Remove the tube from the magnetic rack. Add 10 beads volume (BV) Binding/Wash Buffer to the tube and invert the tube several times to mix. Use the magnetic separation rack to collect the beads and discard the supernatant. Repeat this step twice.
5. Resuspend the beads in Binding/Wash Buffer to create a 25% slurry.

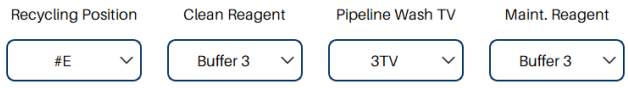
Sample Preparation

1. Resuspend pellets in lysis buffer. We recommend 5 mL/gram of wet pellet.
2. Add beads to the sample and load into the instrument.

ProAb 1300 Setup and Run

1. Setup the protocol (the protocol used in this experiment is below).
2. Create a run by specifying the program, number of samples, and settled beads volume. Confirm the details on the controller.

No.	Operation	Work Pos	Mix CV	Mix Time/s	Mix Speed	Mag Time/s	Waiting/s
1	Transfer	#S	30	7200	Medium	120	0
2	Buffer 1	#A	20	300	High	120	0
3	Buffer 1	#B	20	300	High	120	0
4	Buffer 1	#C	20	300	High	120	0
5	Buffer 2	#D	10	300	High	120	0



3. Load consumables and place the tubing into their corresponding buffers. Buffer 1 is the binding/wash buffer, buffer 2 is the elution buffer, and buffer 3 is MilliQ dH_2O.
4. Confirm the consumables on the controller and begin the run.

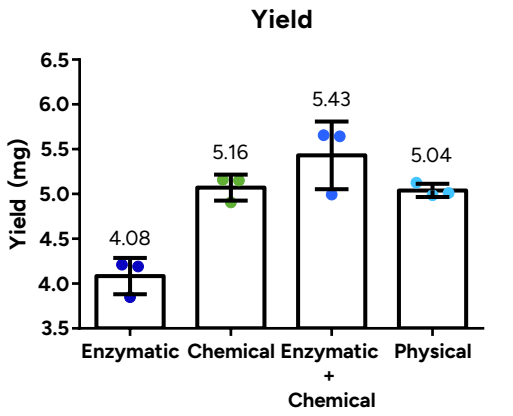
[1] The composition will depend on the lysis method

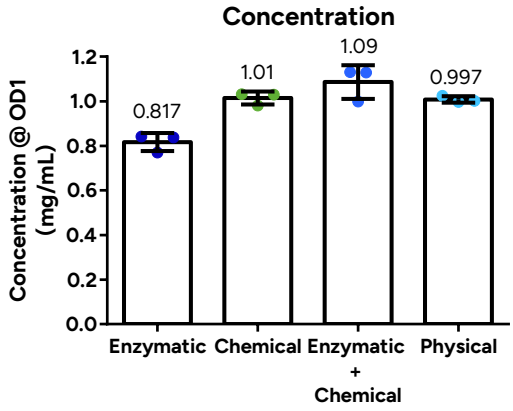
Sample Data

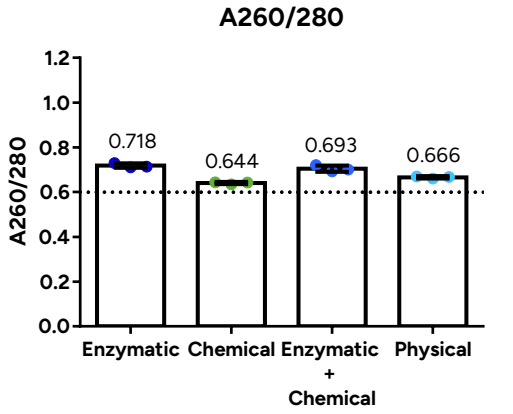
GST-His was purified from *E.coli* using 0.5 mL settled AmMag™ Ni magnetic beads. The samples were analyzed using NanoDrop spectroscopy and SDS-PAGE.

Note: Physical lysis (sonication) was performed as a control: 40% amplitude, 10 s on/off cycles for 3 min on ice.

NanoDrop Analysis







SDS-PAGE Analysis