

HisExpress™ Purification Protocol for Native His-tagged Proteins from *E. coli* Cells (For research use only)

1. Set-up

- a. Place all buffers on ice and allow them to cool. Add protease inhibitors if necessary.
- b. Use tubing tips with the syringe to draw buffers/samples into OmniLyse® cartridge and to dispense sample from HisExpress™ column. Draw 1 mL of Binding buffer into the syringe. Remove the tip and attach the syringe to the OmniLyse® cartridge. Equilibrate the OmniLyse® cartridge by dispensing the Binding Buffer through the cartridge.
- c. Draw 1 mL of Binding Buffer into the syringe. Remove the tip and attach the syringe to the HisExpress™ column. Equilibrate the column by dispensing the Binding Buffer through the column.

2. Cell pellet preparation

- a. Pellet 5 mL of cells in a 15 mL conical tube (50 mL conical for large volumes) by centrifugation at 3000 rpm for 5 min. **A pellet from up to 20 mL of cells can be used with the OmniLyse® cartridge (1x10¹¹ cells).**
- b. Discard supernatant and resuspend pelleted cells with 1 ml Binding Buffer and place on ice. **Smaller volumes (500 µL) and larger volumes can be used. For smaller volumes use a 1 mL syringe.**
- c. Attach a tubing tip to the male luer fitting on the OmniLyse® cartridge and an empty 3 ml syringe to the female luer fitting of the OmniLyse® cartridge. Attach the battery pack to the connector of the OmniLyse® cartridge.

3. Cell Lysis

- a. Draw sufficient sample volume through the tip of the OmniLyse® cartridge to fill the chamber (~ 125 µl) displacing any air. **If during the course of lysis air enters the chamber, turn off the battery pack, dispense the sample and redraw the sample into the chamber displacing the air. Restart the battery pack and continue.**
- b. Turn on the device. Draw the remaining sample through the OmniLyse® cartridge into the syringe. **Be sure that the cartridge chamber remains filled with sample.**
- c. Reverse the direction of the syringe, dispensing the sample back through the cartridge into the original sample container. **Again, the cartridge chamber should remain filled with sample.**
- d. Continue withdrawal and infusion of sample for 3 minutes total (~ 10 to 15 passes).
- e. After the final pass, turn off the device and withdraw the entire lysate into the syringe, emptying the chamber).

4. Purification

- a. Attach the syringe to the HisExpress™ column and dispense the lysate through the column collecting the flow-through in a 1.5 mL microfuge tube on ice.

- b. Attach syringe containing 3 mL of Wash Buffer and dispense through the column. Save for analysis or discard.
- c. Attach syringe containing 1 mL of Elution Buffer and dispense through the column collecting desired sized fractions on ice.

Product use restrictions:

OmniLyse® cartridges and HisExpress™ columns were developed are sold for research purposes only and are suitable for *in vitro* applications only.

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