

HDJ1 PURIFICATION

Materials/Reagents:

LB/amp media (50mL and 1L with each having 100mg/L amp.)
pET-Hdj1 plasmid
BL21/DE3 competent cells
LB/amp plates (2)
IPTG (1mL of 1M)
Lysozyme
Leupeptin and Pepstatin A
Dialysis tubing
TEN₁₀₀ buffer (20mM tris pH 7.4, 0.1mM EDTA, 100mM NaCl)
Ammonium sulfate.
Millipore concentrators.

Procedure:

Overexpression

- 1) (day 1) Transform 100ng of the pET-Hdj1 plasmid into BL21/DE3 competent cells and plate on an LB/amp plate.
- 2) (day 2) Pick a single colony of the transformation and streak several times onto another LB/amp plate.
- 3) (day 3) Pick a single colony off of the streaked plate and place into 50mL LB/amp and incubate overnight at 37°C.
- 4) (day 4) Take the overnight culture and inoculate it to a 40X dilution in 1L LB/amp.
- 5) Grow at 37°C in a shaker until the O.D.₅₉₅ is 0.5-0.9 (around 2 hours).
- 6) Take a 1mL sample and save as an uninduced sample.
- 7) Induce the cells with a final concentration of 1mM IPTG.
- 8) Grow at 30°C in a shaker for 4-6 hours.
- 9) Take a 1mL sample (induced).
- 10) Harvest cells by spinning them down at 5,000 rpm for 20 minutes.
- 11) Remove the supernatant. Pellet can be frozen at -80°C for use later.

Test for overexpression

- 1) Take the uninduced and induced sample and spin at max rpm for 1 minute.
- 2) Aspirate the supernatant.
- 3) Resuspend the pellets in 1x SDS sample buffer (100µL for the uninduced and 200µL for the induced)
- 4) Load 10µL each on a SDS-PAGE gel.
- 5) Look for overexpression at the predicted size. There should be a much larger band in the overexpression lane.

Cell lysis

- 1) Resuspend cells in Ten₁₀₀ buffer at 3ml per 1g of cells.
- 2) Add lysozyme to 0.5mg/mL concentration.
- 3) Add leupeptin and pepstatin A to concentrations of 1µg/µL.
- 4) Put on a nurator in the cold room for 30 minutes.
- 5) Freeze and thaw 4X in a methanol bath and 37°C water bath.
- 6) Sonicate 30X in 3X cycles (using 30-40% duty cycle and 3-4 output control) or until the viscosity is greatly reduced.
- 7) Centrifuge at 32000rcf for 60 minutes
- 8) Take the supernatant.

Purification

- 1) Add 0.28g of ammonium sulfate (powdered with a mortar and pestle) per mL of lysate and incubate at 4°C for 30 minutes with slow stirring.
- 2) Spin the mixture down at 14,000rcf for 30 minutes.
- 3) Throw away the supernatant and resuspend the pellet in 15mL TEN₁₀₀ buffer.
- 4) Perform dialysis in 2L of TEN₁₀₀ buffer for 4 hours.
- 5) First separate the lysate over a DEAE column using a salt gradient from 0mM NaCl to 500mM NaCl over 1000mL of elution.
- 6) Run gels with uninduced and induced samples along with 16µL of each odd fraction.
- 7) Stain by coomassie and identify fractions containing Hdj1.
- 8) Pool fractions.
- 9) Next resolve over the mono S column using a Hapes (20mM pH 7.4) buffer using an elution volume of 20mL and salt gradient from 0mM NaCl to 500mM NaCl.
- 10) Run odd samples on a gel as described before and identify fractions that contain Hdj1.
- 11) Concentrate the protein using Millipore concentrators.

Troubleshooting/Critical Parameters:

If overexpression is not achieved, try lowering the overexpression temperature. Also, the overnight and initial dilutions can be performed at lower temperatures. IPTG concentration can also be increased. The indicated parameters should work. See protocols for FPLC use and programming for column help.

References:

Zylicz, M., Yamamoto, T., McKittrick, N., Sell, S., and Georgopoulos, C. *J. Biol. Chem.*, **260**: 7591-7598 (1985).

Submitted by:

Brandon Toyama