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# Small-scale Expression and Solubility Testing of Proteins in BL21 *E. coli*

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## ABSTRACT

Scaled-down solubility screen for bacterial overexpression constructs.

## THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Modified from Savitsky et al 2010

## ATTACHMENTS

[1-s2.0-S1047847710001863-main.pdf](#)

## PROTOCOL CITATION

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**protocols.io**  
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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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## CREATED

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Jul 17, 2020

## PROTOCOL INTEGER ID

36106

## GUIDELINES

Use standard aseptic techniques and proper PPE. Dispose of waste and used culture medium as appropriate.

## MATERIALS TEXT

### Lysis Buffer

50 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 7.5-8.0)

500 mM NaCl

0.1% Triton X-100

10 mM imidazole

1 mg/mL lysozyme

10 uM Beta-mercaptoethanol

1 EDTA-free Protease inhibitor tablet/10mL buffer

#### Wash Buffer

50 mM NaPO<sub>4</sub> (pH 7.5-8.0)

500 mM NaCl

30 mM imidazole

#### Elution Buffer

50 mM NaPO<sub>4</sub> (pH 7.5-8.0)

500 mM NaCl

250 mM imidazole

TALON resin (Takara)

#### SAFETY WARNINGS

BME can smell noxious and be an irritant, so use a chemical hood when preparing stock for Lysis Buffer.

#### BEFORE STARTING

Make sure you have fresh buffers and keep samples on ice when noted.

#### Screening

- 1 Grow and induce BL21 cells in medium of your choice with appropriate temperature and inducer conditions. We often use Terrific Broth and culture cells at 30 °C, with induction carried out at 18 °C overnight (1mM IPGTG or 0.5% L-arabinose).
- 2 Harvest 10 mL overnight expression culture by centrifugation for 10 minutes at 3000 x *g*.
- 3 Remove supernatant by decanting and resuspend pellet in 800 uL Lysis Buffer. Transfer cell suspension to a new 1.5 mL Eppendorf tube and store on ice.
- 4 Sonicate as appropriate for complete cell lysis (settings will depend on make and model of your sonicator).
- 5 Clarify lysates by centrifugation at 4 °C for 10 minutes at 12-15000 x *g*.

- 6 While lysates are spinning, equilibrate the Talon resin by washing 3x with 5 volumes of resin bedding.

NOTE: For each pulldown you do, you will need 25  $\mu$ L of equilibrated Talon resin. This comes supplied as a 50% slurry in ethanol, so calculate how many pulldowns you will be doing and then add 50  $\mu$ L. If you have 8, estimate you will need enough for 10 pull-downs. To equilibrate, shake the slurry well and remove 500  $\mu$ L resin slurry with pipette. Transfer slurry to new Eppendorf tube. Pellet resin by spinning in microfuge for 30 seconds at 3000 x *g*, then remove supernatant carefully and add 1 mL of Lysis Buffer. Vortex resin briefly, then pellet again and wash beads 2x more with 1 mL of Lysis Buffer. After the third spin, remove supernatant and add 250  $\mu$ L of Lysis Buffer to produce 500  $\mu$ L of equilibrated resin.

- 7 Transfer 750  $\mu$ L of clarified supernatant to a new Eppendorf tube.
- 8 Add 50  $\mu$ L equilibrated resin to each Eppendorf tube and incubate for 1 hr at RT with end-over-end mixing.
- 9 After 1 hour, pellet resin with 1 min 3000 x *g* spin at RT, then remove supernatant with pipette.
- 10 Wash resin 3x with 1 mL of Wash Buffer, pelleting resin between each wash and removing supernatant.
- 11 After third wash, remove supernatant and resuspend resin in 50  $\mu$ L of Elution Buffer.
- 12 Incubate at RT for 5-10 minutes, then quickly spin down beads and transfer eluate to clean Eppendorf tube.
- 13 Repeat elution again, combining eluates in single tube at the end (100  $\mu$ L eluate).
- 14 Analyze 5-10  $\mu$ L of eluate on an SDS gel and stain with Coomassie blue.