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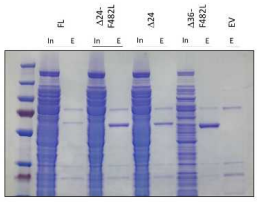
Small-scale Expression and Solubility Testing of Proteins in BL21 E. coli



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We use this protocol and it's working

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Abstract

Scaled-down solubility screen for bacterial overexpression constructs.

Attachments

PDF

1-s2.0-S104784771000...

796KB

Guidelines

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

Materials

Lysis Buffer

50 mM NaH_2PO_4 (pH 7.5-8.0)

500 mM NaCl
0.1% Trition 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 $NaPO_4$ (pH 7.5-8.0)

500 mM NaCl
30 mM imidazole

Elution Buffer

50 mM $NaPO_4$ (pH 7.5-8.0)

500 mM NaCl
250 mM imidazole

TALON resin (Takara)

Troubleshooting

Safety warnings

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

Before start

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

Screening

1 Grow and induce BL21cells in medium of your choice with appropriate temperature and inducer conditions. We often use Teriffic Broth and culture cells at 30 ^{O}C, with induction carried out at 18 ^{O}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 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 ^{O}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 ul 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 uL. If you have 8, estimate you will need enough for 10 pull-downs. To equilibrate, shake the slurry well and remove 500 ul 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 uL of Lysis Buffer to produce 500 uL of equilibrated resin.

7 Transfer 750 uL of clarified supernatant to a new Eppendorf tube.

8 Add 50 uL 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 piepette.

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 uL 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 uL eluate).

14 Analyze 5-10 uL of eluate on an SDS gel and stain with Coomassie blue.