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E. coli protein expression and purification

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ABSTRACT

Protocol for recombinant protein expression in E. coli for enzyme assays, protein crystallography etc.




GUIDELINES

This protocol will take a few days so be sure to have all buffers, cell strains, and plasmids on hand. It can be tricky to organise, practice and familiarity are the best tools. Different sections do not need to be performed immediately after each other - there are various safe stopping steps where cells can be stored at -20/-80 °C until you are ready to continue with the protocol. However, for convenience, the entire protocol is described here.

Adjust volumes, taking care to ensure appropriate vessels are used to allow proper aeration (e.g. Grow 800mL culture in 2L flasks, 1.3L culture in 3L flasks and 2L culture in 5L flasks), depending on the desired downstream application and expected protein yield (e.g. trialling expression vs bulk production for crystallography). We commonly use BL21 (DE3) strains for T7 expression (i.e. IPTG induction).

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
Potassium chloride	View	P212121
Petri Dish	LI-PD01100	P212121
Luria-Bertani (LB) broth, makes 1L	K488	Amresco
EDTA		
1.5 mL Eppendorf tubes		
Electroporation System Gene Pulser XCell		Bio-rad Laboratories
37°C Incubator		
DTT	D0632	Sigma Aldrich
2.5ml SDS-PAGE Sample Loading Buffer [2X]	786-025	G-Biosciences
14ml Polystyrene Cell Culture Tubes	CT5250	Alkali Scientific
4X Bolt LDS Sample Buffer	B0007	Invitrogen - Thermo Fisher
NaCl	53014	Sigma Aldrich
Heat transfer block	Z3271	Promega
IPTG	IB0168.SIZE.100g	Bio Basic Inc.
Coomassie Blue Staining & Destaining Solution	AR0140	Boster Bio
BL21(DE3) or BL21-Star(DE3) or Rosetta2(DE3) or etc for protein purification		
Magnesium chloride hexahydrate	M2670	Sigma Aldrich

NAME 	CATALOG # 	VENDOR 
Electroporation Cuvette 1mm	1652089	BioRad Sciences
Falcon® Conical Tubes, 50 mL 500 Tubes	38010	Stemcell Technologies
Tris-HCl	AM9855	Life Technologies
28°C incubator without CO2		Thermo Fisher Scientific
Disodium phosphate	S7907	Sigma Aldrich
Monopotassium phosphate	P9791	Sigma Aldrich
42°C water bath		
Imidazole	I5513	Sigma Aldrich
UV/Vis spectrophotometer	View	
GelCode™ Blue Stain Reagent	24590	Thermo Fisher Scientific

SAFETY WARNINGS

Ensure use of appropriate aseptic technique. Use caution if using a bunsen burner and ethanol.

BEFORE STARTING

Make sure you have your verified plasmid transformed into your desired E. coli strain for protein expression e.g. BL21 Star (DE3).

These should be plated on selective LB media to produce positive colonies for starter cultures. Prepare all the buffers described in Step 1, except make fresh IPTG stocks (ideally).

Prepare buffers

1 10X PBS:

- Dissolve the following in 800ml distilled H₂O.
 - 80 g of NaCl
 - 2.0 g of KCl
 - 14.4 g of Na₂HPO₄
 - 2.4 g of KH₂PO₄
- Adjust pH to 7.4.
- Add H₂O to 1L.
- Autoclave

Store 10X stock at 4 °C from which you can dilute 1:10 to make 1X working stock to keep at room temp.

Resuspension buffer:

50 mM Tris-HCl pH 8
2 mM EDTA

1X Binding Buffer:

50 mM NaH₂PO₄, pH 8
300 mM NaCl
10 mM Imidazole

1X Wash Buffer:

50 mM NaH₂PO₄, pH 8
300 mM NaCl
20 mM Imidazole

1X Elute Buffer:

50 mM NaH₂PO₄, pH 8
300 mM NaCl
250 mM Imidazole

Digestion / Storage buffer:

50 mM Tris-HCl pH 8
150 mM NaCl
20 mM KCl

Transformation

- 2 Transform desired E. coli cell strain with plasmid to be expressed using desired method (e.g. heat shock or electroporation depending on type of competency).
- 3 For electrocompetent cells:
 - Add 0.5 - 1 µL purified plasmid to 50 µL cells (thawing on ice, 15 minutes)
 - Gently flick with finger to mix
 - Transfer mixer to chilled electroporation cuvette ensuring there are no bubbles. Keep on ice until ready to electroporate
 - Set machine to 1.8 kV, 25 µF, 200-400 Ω
 - Dry the outside of the cuvette and place into electroporation chamber.
 - Prepare p1000 and p200 pipettes to be ready
 - Close chamber and electroporate
 - Immediately remove cuvette and add 1 mL LB. Transfer contents to microfuge tube using both p1000 and p200.
 - Let cells recover at 37 °C with ~200 rpm shaking for > 1 hour.

14h

- 4 For chemically competent cells:
 - Add 0.5 - 1 μ L purified plasmid to 50 μ L cells (thawing on ice, 15 minutes)
 - Gently flick with finger to mix
 - Sit on ice for 30 minutes, set water bath to heat to 42 $^{\circ}$ C
 - Depending on cells, incubate in water bath for 30 - 90 seconds.
 - Return to ice for 5 minutes
 - Add 1 mL LB and let cells recover at 37 $^{\circ}$ C with \sim 200 rpm shaking for > 1 hour.
- 5 Plate recovered transformed cells (\sim 100 μ L of transformed cells) onto selective LB media and grow O/N @ 37 $^{\circ}$ C. Adjust volume as needed in order to obtain single colonies that can be picked for subsequent inoculation.

1h

Protein expression

- 6 Inoculate bacterial colony from selective media into LB + antibiotic of choice. Use p100-200 pipette tip to scrape colony and drop into 3-5 mL broth in 10 mL culture tube. Grow O/N @ 37 $^{\circ}$ C with \sim 200 rpm shaking.
- 7 Inoculate larger culture using the starter culture generated from Step 3 at 1:100 dilution (e.g. 0.5 mL in 50 mL LB + antibiotic). Larger culture volume will depend on what you plan to do with the protein. For example, for large-scale protein production you may use 1 - 2 L cultures - ensure you use an appropriate vessel to allow appropriate aeration (see suggestions in guidelines). For smaller scale tests for protein induction and solubility, you may prefer 10 - 15 mL cultures in 50 mL falcon tubes.
- 8 Grow larger culture at 37 $^{\circ}$ C and check OD₆₀₀ after 2.5 - 3 hours (times may vary depending on total culture volume and quality of cells).
- 9 When OD₆₀₀ is between 0.6 - 0.8, take an aliquot of culture (up to 15 mL) as non-induced control. To the remaining culture, add induction media. We typically use BL21 (DE3) derived strains and, thus, add 100 mM IPTG to achieve [IPTG]_{final} = 1 mM (i.e. 1:100 dilution, 100 μ L IPTG to 10 mL culture).
- 10 Grow cultures for approx. 5 hours, then check OD for difference between induced vs non-induced. Non-induced should be higher by at least 0.1.

1h

1m

3h

1m

QC protein induction

- 11 If you are running small scale test induction (e.g. testing constructs, induction, protein solubility), we can run SDS-page gels on the crude lysate (\sim 1-2 mL culture) before trying larger cultures. With larger cultures, you may want to take an aliquot of the culture to check induction before proceeding with purification. If you are confident, proceed to step 21 for washing and storage.
- 12 Spin down culture (\sim 1 - 3 mL) at max speed for 3 minutes and remove supernatant. This pellet can be stored O/N @ -20 $^{\circ}$ C until you are ready to proceed with checking induction of your expressed recombinant protein.
- 13 Resuspend cells in 100 μ L 1X PBS (per 1 mL culture).
- 14 Calculate how much crude lysate to load onto gel based on OD (μ L = 180/OD).
- 15 To these volumes, add the appropriate amount of 4X LDS (or required SDS-PAGE sample buffer), DTT (final concentration = 50 mM) and MgCl₂ (final concentration = 100 mM). These should be calculated first, and a mastermix of LDS, DTT and MgCl₂ can be prepared and added to samples accordingly.

2m

1m

1m

- 16 Heat sample @ 72 °C for 10 minutes on heat transfer block. 1m
- 17 Place samples on ice for 5 minutes, then spin for 15 minutes max speed. 2m
- 18 Transfer supernatant to new tubes, taking care to avoid any "sticky" DNA coating the tube.
- 19 Run supernatant on SDS-PAGE gel (160V, 40-45 minutes).
- 20 Stain gel with Coomassie or Gel Code Blue (or other stain of choice). Alternatively, perform Western blot if antibodies are available or recombinant proteins are epitope tagged (e.g. MYC).

Store cells for purification

- 21 If you are confident with your protein induction (see QC) and want to preserve the cell culture for protein purification, spin down cells at 4 °C (~7000 rcf for 5 minutes with gentle/no brake). Remove supernatant. 1m
- 22 Wash cells in resuspension buffer, spin down and remove supernatant.
- 23 Snap-freeze pellet in LN₂ and store at -80 °C.

Likely, this will be done pre-emptively until results from the QC are obtained, then cells can be discarded or retained accordingly.

This is a safe stopping point where you can continue with protein purification as needed.

Protein purification

- 24 Take stored cells out of -80 °C and let thaw on ice. 3m



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