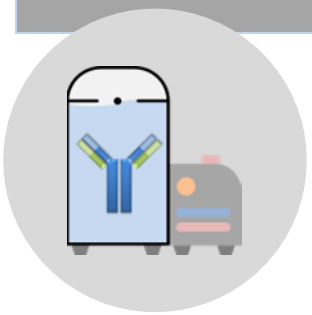

Recombinant Protein Expression

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

Recombinant protein expression means the production of a foreign protein in an organism like bacteria or fungi. In this protocol, we describe the expression of proteins in *E. coli*, a widely used bacterium for protein expression purposes. It's important to note that each protein is unique and different DNA constructs may require specific conditions such as antibiotics, bacterial strains, temperatures, incubation times or media. The protocol provided here offers a standard procedure for IPTG-inducible promoters, such as the *lac*-promoter. It may be necessary to adapt the protocol to suit the specific requirements and optimize the expression of the desired protein.

Material		
Bacteria strain for expression	*E.coli* expression strain	
LB (Lysogeny Broth)	0.5 % (m/v) Yeast Extract 1 % (m/v) Tryptone 1 % (m/v) NaCl Selection Antibiotic	
Inductor	IPTG (Isopropyl-β-D-thiogalactopyranosid)	https://davids-science.de/p/iptg
Lysis Buffer	100 mM NaH_2PO_4 10 mM Tris-HCl 6 M Guanidine-HCl pH 8.0	
Wash Buffer	100 mM NaH_2PO_4 10 mM Tris-HCl 6 M Guanidine-HCl pH 6.6	
Elution Buffer	100 mM NaH_2PO_4 10 mM Tris-HCl 6 M Guanidine-HCl pH 4.0	

- 2 - Method

Culture Preparation

- Inoculate 5 – 15 ml of LB media with your desired *E. coli* strain
  You can pick a single colony from your agar plate and transfer it to the media. Note that optimal growth is achieved when using a flask with only 1/3 of the medium volume (e.g., 10 ml medium in a 30 ml culture flask)
- Add antibiotics to the media if necessary
- Incubate the culture at 37°C and 180 rpm overnight
- Inoculate 500 ml of preheated LB media with 1 ml of the overnight culture
- Add antibiotics to the media if necessary
- Incubate the bacteria culture at 37°C and 180 rpm

Induction

- Check the optical density of your culture frequently at 600 nm (OD_{600})
- Induce the culture with IPTG at a final concentration of 1 mM when the OD_{600} reaches 0.6
  The incubation time required to achieve an OD_{600} of 0.6 may vary for each culture. Typically, the optical density for induction is obtained after 2 to 4 hours.

Cultivation

- Incubate the culture at 22°C overnight
- Centrifuge the bacteria culture at 3,200 x g for 20 minutes
- Discard the supernatant carefully
- Wash the bacteria pellet once with 1x PBS
- Centrifuge the cells again after washing and discard the buffer
- Freeze the bacteria pellet at -20°C

Protein Isolation

- Thaw the frozen cell pellet by placing it on ice for 15 minutes
- Resuspend the thawed cell pellet in 4 ml of Lysis Buffer
- Incubate the cell mixture under gentle shaking for 1 hour
- Centrifuge the cell lysate at 10,000 x g for 20 minutes
- Utilize the supernatant obtained from the centrifugation step for further protein purification
  Backup the resulting pellet for further protein isolation. The pellet may contain insoluble proteins or other components that could be targeted for purification through alternative methods.

Protein Purification

- Utilize a compatible chromatography column with a bed volume of 2.5 ml
  Please refer to the manual of the specific resin you are using, as different resins require different handling procedures. The following steps outline a general procedure, but it may need to be adjusted according to your specific resin and protein construct
- Equilibrate the column by passing through 5 column volumes of Lysis Buffer
- Recirculate the protein supernatant through the column at a flow rate of 1 ml/minute for 2 hours
- Rinse the column with 20 column volumes of Wash Buffer
- Elute the purified protein by passing through 6 column volumes of Elution Buffer

- 3 - Trouble Shooting

Reason	Solution
Low Viability	Ensure the LB media is preheated before inoculation to provide optimal growth conditions for the cells.
Antibiotic	Confirm that you are using the appropriate antibiotic that matches the expression vector being used. Check the compatibility and concentration required for effective selection.
Expression Efficiency	Reduce the final concentration of IPTG used for induction to a range of 0.1 to 0.5 mM to optimize protein expression levels.
Low Production	Reduce the optical density of the culture to a range of 0.4 to 0.6 at 600 nm just before induction. This ensures a favorable starting point for protein production.
Low Yield	Enhance protein expression by adding 2% ethanol (v/v) to the LB media before induction. Ethanol can improve protein yield in certain cases.
Inclusion Bodies	For intracellular proteins that tend to form inclusion bodies, additional isolation methods such as ultrasonic treatment may be required.
Toxic Proteins	Reducing the cultivation temperature of induced cells to 16°C can mitigate the potential toxic effects of expressed proteins.
Protein Resolution	Change the elution method from isocratic to gradient elution. A gradient elution approach can improve protein resolution and separation during the purification process.
Long Time Storage	For long-term storage of the purified protein it is recommended to dialyze the protein sample against 1x PBS. This step helps remove small molecules and unwanted buffer components, ensuring the protein is in a suitable storage buffer.