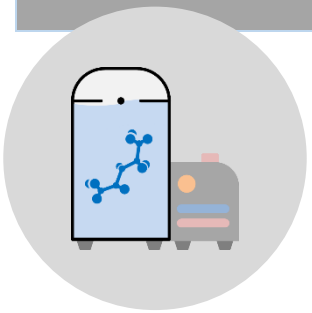

Recombinant Protein Expression

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

- 1 - Introduction

Recombinant protein expression stands for the production of a foreign protein in an organism. In this protocol we describe the expression in *E. coli*, a very common bacteria for the expression. Each protein is different and each DNA construct may need special conditions like antibiotics, bacteria strains, temperatures, incubation times or media. With this protocol you receive a standard that can be used for IPTG induceable promotors (i. e. lac-promotor). You may need to adapt it to your special needs.

- 2 - Material

Material		
Bacteria strain for expression	*E. coli* expression strain	
LB Media	0.5 % (m/v) Yeast Extract 1 % (m/v) Tryptone 1 % (m/v) NaCl Selection Antibiotic	
Inductor	IPTG	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	

- 3 - Method

Preparation of an *E. coli* culture

- Inoculate 5 – 15 ml LB media with your desired *E. coli* strain
- You may want to pick one colony from your agar plate for inoculation. Please keep in mind that best growth is performed in a flask with only 1/3 of medium (i. e. 10 ml medium in a 30 ml culture flask).
- Add antibiotics if necessary
- Incubate at 37°C, 180 rpm over night

Cultivation

- Inoculate 500 ml preheated LB media with 1 ml overnight culture
- Add antibiotics if necessary
- Incubate the bacteria culture at 37°C, 180 rpm

Induction

- Check the optical density of your culture frequently at 600 nm (OD_{600})
- Induce with IPTG with a final concentration of 1 mM at OD_{600} = 0.6
- The incubation time to achieve an OD_{600} of 0.6 is different for each culture. Usually, you will achieve the best OD_{600} after 2 – 4 hours when the temperature is 37°C.

Cultivation (II)

- Incubate the culture at 22°C over night
- Centrifugate bacteria at 3.200 x g for 20 minutes
- Discard the supernatant
- You may want to wash the pellet once with 1 x PBS or another physiological buffer
- Freeze the bacteria pellet at – 20°C

Protein Isolation

- Thaw the frozen cell pellet on ice for 15 minutes
- Resuspend the pellet in 4 ml of Lysis Buffer
- Incubate the cell mixture under gently shaking for 1 hour
- Centrifugate at 10.000 x g for 20 minutes
- Utilize the supernatant for further protein purification
- *You may want to backup the resulting pellet for further protein isolation*

Protein Purification

- *Please have a look at the manual of the resin you use for protein purification. Each resin needs different handlings. The following procedure may not work with your specific construct.*
- Utilize 2.5 ml of a compatible chromatography column
- Equilibrate column with 5 column volume of Lysis Buffer
- Recirculate protein supernatant with a flowrate of 1 ml/minute for 2 hours
- Rinse the column with 20 column volume of Wash Buffer
- Elute the protein with 6 column volume of Elution Buffer
- *You may want to dialyze against 1 x PBS your protein sample for long time storage*

- 4 - Trouble Shooting

Reason	Solution
Low Viability	Ensure preheated LB media
Antibiotic	Ensure right antibiotic for matching expression vector
Expression Efficiency	Reduce final concentration of IPTG between 0.1 to 0.5 mM
OD_{600}	When you missed the optimal spot of OD_{600} = 0.6, you can usually achieve good results with OD_{600} between 0.4 – 1.
Low Production	Reduce optic density right before induction to 0.4 – 0.6 at 600 nm
Low Yield	Enhance protein expression by adding 2 % ethanol (v/v) to LB media before induction
Inclusion Bodies	For intracellular proteins additional isolation methods like ultrasonic treatment may be required
Toxic Protein Expression	Reduce cultivation of induced cells temperature to 16 °C over night
Protein Resolution	Change Elution method from isocratic to gradient elution