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Small Scale IPTG Induction | Protein Expression

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Test

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Disclaimer

> Test

Abstract

Test

Before start

It's best to start small-scale protein purification on a Monday or Tuesday. This protocol will take at least 3 days before the first freezing step.

Seed Culture

1d

1 Preheat the shaking tube incubator to 37 °C

2 Label a 15 mL culture tube with the vector strain you will be using, and fill with 5 mL of LB broth with 1x Kanamycin antibiotic or 5ul of 1000x.

3 Place the labeled tube into the shaking incubator to warm, then grab an ice bucket and fill with ice.

4 Take the BL21 glycerol stock from the -80 °C freezer and place immediately into the ice bucket.

5 Use a 200 µL pipette tip to scrape some of the ice from the tube, and place the tip directly into the culture tube with LB.

6 Let the culture shake at 37 °C overnight to be completely saturated by the next morning.

12h

Culture Expansion

2h

7 Grab a sterilized 250 mL Erlenmeyer flask and fill with 45mL of Terrific Broth and 1x Kanamycin antibiotic.

8 Prewarm the flask in the shaking tube incubator at 37 °C

9 Add all 5 mL of the seed culture to the Erlenmeyer flask. The culture should expand 1:10 in 1-2 hours, to an OD around 0.4-0.6.

1h 30m

10 Thaw a stock of IPTG on ice about 30 minutes before you suspect the culture will be at 0.4 OD.

30m

11 Quantify the OD of your 50 mL flask using 500 µL of culture in a spectrometer cuvette. Be sure to blank with 500 µL of Terrific Broth.

12 Once OD hits 0.4-0.6, preheat the fridge shaking incubator to 16 °C

13 Dilute [M] 1 Molarity (M) IPTG stock to the appropriate concentration for protein induction by adding to expanded culture. This will most likely be a range of [M] 100 micromolar (µM) to [M] 1 millimolar (mM)

13.1 To calculate IPTG concentration, use V1 x C1 = V2 x C2, where column E is equivalent to V2.

	A	B	C	D	E
	50mL	1mM	**?**	1000mM	**50uL**
	50mL	0.8mM	**?**	1000mM	**40uL**
	50mL	0.6mM	**?**	1000mM	**30uL**
	50mL	0.4mM	**?**	1000mM	**20uL**
	50mL	0.2mM	**?**	1000mM	**10uL**

14 Take your IPTG-induced culture and shake overnight at 16 °C for about 16-18 hours. 18h

Pellet Cells 5m

15 Take each 50 mL culture and pour into a 50 mL conical tube, labelled.

16 Take the cells to a large or spinning-bucket centrifuge and spin at 500 x g, 4°C, 00:05:00 5m

17 Decant cells into a bleach waste bin labeled for culture, and dry on a paper towel for 5-10 seconds 10s

18 Freeze the pellet at -80 °C by storing in a freezer for storage, or continue to the protocol for cell lysis.