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Preparation of Autoinduction Lysogeny broth (LB) for Protein Expression

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Protocol status: Working

We use this protocol and it's working

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Abstract

Auto-induction LB (ZYM-5052) enables high-level recombinant protein expression in *E. coli* without manual IPTG induction. This protocol provides a streamlined recipe and culture workflow adapted from Studier (2005) for T7-based systems (e.g., pET vectors in BL21(DE3) and derivatives). Medium is assembled from sterile concentrates: 20× NPS salts, 50× "5052" carbon mix (glycerol, glucose, lactose), 1 mM MgSO₄, and appropriate antibiotic(s). Media used in baffled flasks at ≤20–25% fill to ensure aeration. Glucose supports initial growth and represses expression; as it is exhausted, lactose automatically induces T7 RNA polymerase while glycerol sustains continued growth.

Materials

Expression Lysogeny Broth (LB) 1 L

- Tryptone  10 g (1% of Autoinduction final volume)
- Yeast Extract  5 g (0.5% of Autoinduction final volume)
- Ultra-pure water up to  930 mL

5052 Solution (50x) 500 mL

- Glycerol  125 mL (25%)
- Glucose  12.5 g (2.5%)
- α-Lactose  50 g (10%)
- Ultra-pure water up to  500 mL

Nitrogen-Phosphate Salts (NPS; 20x) 1 L

- Ammonium Sulfate $(\text{NH}_4)_2\text{SO}_4$  66.07 g  0.5 Molarity (M)
- Monopotassium phosphate KH_2PO_4  174.2 g  1 Molarity (M)
- Disodium phosphate Na_2HPO_4  141.96 g  1 Molarity (M)
- Ultra-pure water up to  1 L

Magnesium Sulphate 50 mL 1 Molarity (M)

- Magnesium Sulphate MgSO_4  12.32 g  1 Molarity (M)
- Ultra-pure water up to  50 mL

Antibiotics

 1 mL of filter-sterilised selected antibiotics at stock concentration.

Troubleshooting



Materials

1 **Expression Lysogeny Broth (LB)** 1 L

- Tryptone  10 g (1% of Autoinduction final volume)
- Yeast Extract  5 g (0.5% of Autoinduction final volume)
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- Ultra-pure water up to  1 L

4 **Magnesium Sulphate** 50 mL [M] 1 Molarity (M)

- Magnesium Sulphate MgSO_4  12.32 g [M] 1 Molarity (M)
- Ultra-pure water up to  50 mL

5 **Antibiotics**

 1 mL of filter-sterilised selected antibiotics at stock concentration.

 [go to step #10](#) for common stock concentrations.

Preparation of Components

1h

6 **Preparation of Expression LB**

Add all dry ingredients to a  1 L measuring cylinder

6.1 Add ultra-pure water up to  930 mL



6.2 Add magnetic stir bar and mix thoroughly

6.3 Add mixture to  1 L Duran bottle, place lid loosely, and autoclave to sterilise with settings:  00:20:00  121 °C

20m

7 **Preparation of 5052 Solution (50x)**

Add dry ingredients to a  1 L measuring cylinder

7.1 Add glycerol to a  500 mL measuring cylinder

7.2 Add ultra-pure water up to  500 mL

7.3 Add mixture to  500 mL Duran bottle, place lid loosely, and autoclave to sterilise with settings:  00:20:00  121 °C

20m

8 **Preparation of NPS (20x)**

Add dry ingredients to a  1 L measuring cylinder

8.1 Add ultra-pure water up to  1 L

8.2 Add magnetic stir bar and mix thoroughly

8.3 Add mixture to  1 L Duran bottle, place lid loosely, and autoclave to sterilise with settings:  00:20:00  121 °C

9 **Preparation of Magnesium Sulphate**

Add magnesium sulphate to a  50 mL measuring cylinder

9.1 Add ultra-pure water up to  50 mL

9.2 Add magnetic stir bar and mix thoroughly

9.3 Add mixture to  50 mL Duran bottle, place lid loosely, and autoclave to sterilise with settings:  00:20:00  121 °C

20m

Making Autoinduction LB Broth

10 After letting all solutions cool to  Room temperature :



Add to a  5 L baffled flask:

-  1 mL of [M] 1 Molarity (M) Magnesium Sulphate
-  1 mL of appropriate antibiotic

	A	B	C
		Stock Concentration	Final Concentration
	<i>Ampicillin</i>	100 mg/mL	100 µg/mL
	<i>Chloramphenical</i>	25 mg/mL	25 µg/mL
	<i>Kanamycin</i>	25 mg/mL	25 µg/mL

-  50 mL of 20x NPS
-  20 mL of 50× 5052 Solution
- 1 bottle of Expression LB ( 930 mL)

11 Alternatively, measure out appropriate volumes of autoinduction media into other sizes of baffled flasks:



	A	B
	Media Volume (mL)	Flask Size (mL)
	125	500
	250	1000



	A	B
	500	2000

Media to flask ratio generally 1:4

Inoculating Autoinduction Broth

12 Add inoculate  1 mL of starter culture to begin expression in desired conditions

Protocol references

Studier, F.W. (2005) Protein Expression and Purification 41:207–234.