

Auto induced media preparation

Based on F.W. Studier / Protein Expression and Purification 41 (2005) 207–234

Procedure

1. Pick one colony of freshly transformed bacteria into 10ml snap-cap tube containing 1-5ml auto-induced media
2. Grow over night (maximum 16h) in shaker at 37C and 30C.
3. Spin down 1.5ml aliquots in eppendorf tubes and aspirate media.
4. Freeze the dry pellets
5. Lyse the pellets in lysis buffer, sonicate, and run sup vs. pellet fractions for coomassie and western blot analysis (instructions for this section can be found here:
<http://wolfson.huji.ac.il/expression/procedures/bacterial/Induction.Condition.Callib.new.htm>

- Multiple bacterial strains and media composition can be tested in parallel, using a 96 or 48 deep-well plates containing 0.5ml-1ml medium . (Please note that the shaker's RPM should be changed according to the deep-well type)



Composing the Auto-Induced media:

Materials

Formulation of ZYP-5052 : rich medium for auto-induction (this protocol is based on ZY media and it can be used with any other media, you can replace the ZY media with LB, TB or 2xYT for additional screening)

For all media, add 1M MgSO₄ & 1000x metals mix before adding 20xNPS to avoid precipitate.

	<u>1 litter total</u>	<u>final conc.</u>
ZY (or 2xYT, LB etc.)	~928 ml	
1M MgSO ₄	1 ml	1mM
50x5052	20 ml	1x
20xNPS	50 ml	1x
antibiotic, <u>as needed</u> :		

kanamycin (25 mg/ml)	4 ml	100ug/ml
chloramphenicol (25 mg/ml)	1 ml	25 ug/ml
ampicillin (50 mg/ml)	1 ml	50 ug/ml

Stock solution:

Use ddW for all solutions. Autoclave solutions for 15 min unless specified otherwise.

ZY

10 g bacto tryptone
5 g yeast extract
925 ml water

20xNPS: (NPS= 100mM PO₄, 25mM SO₄, 50mM NH₄, 100mM Na, 50mM K)

To make 100 ml:

90 ml water

6.6 (NH₄)₂ SO₄ =0.5M

13.6 g KH₂PO₄=1M

14.2 g Na₂HPO₄=1M

add in sequence in beaker, stir until dissolve. pH of 20 fold dilution in water ~6.75.

50x5052: (5052= 0.5% glycerol, 0.05% glucose, 0.2% α-lactose)

To make 100 ml:

25 g glycerol (weigh in beaker)

73 ml water

2.5 g glucose

10 g α-lactose

add in sequence in beaker, stir until dissolve. can speed up by heating in microwave.

1M MgSO₄

24.65 g MgSO₄·7H₂O

water to make 100 ml