

New Studier media for auto-induction and other applications

Reference: F.W. Studier (2005) Protein production by auto-induction in high-density shaking cultures. *Prot. Exp. Pur.* 41, 207-234.

ZYM-5052 medium for auto-induction

It is recommended to use this medium for auto-induction. The amount of kanamycin (when appropriate) used should be 100 $\mu\text{g/ml}$.

958 ml	ZY	tryptone	1%
		yeast extract	0.5%
20 ml	50 x M	Na_2HPO_4	25 mM
		KH_2PO_4	25 mM
		NH_4Cl	50 mM
		Na_2SO_4	5 mM
20 ml	50 x 5052	glycerol	0.5%
		glucose	0.05%
		α -lactose	0.2%
2 ml	1 M MgSO_4	MgSO_4	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

ZYP-5052 medium for auto-induction

This medium should be used when stronger buffering is needed. The amount of kanamycin (when appropriate) used should be at least 200 $\mu\text{g/ml}$. Better even would be 400 $\mu\text{g/ml}$.

928 ml	ZY	tryptone	1%
		yeast extract	0.5%
50 ml	20 x P (formerly NPS)	Na_2HPO_4	50 mM
		KH_2PO_4	50 mM
		$(\text{NH}_4)_2\text{SO}_4$	25 mM
20 ml	50 x 5052	glycerol	0.5%
		glucose	0.05%
		α -lactose	0.2%
2 ml	1 M MgSO_4	MgSO_4	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

ZYM-505 medium for plasmid preparation

This medium is used to grow high-density cultures for plasmid preparations.

968 ml	ZY	tryptone	1%
		yeast extract	0.5%
20 ml	50 x M	Na ₂ HPO ₄	25 mM
		KH ₂ PO ₄	25 mM
		NH ₄ Cl	50 mM
		Na ₂ SO ₄	5 mM
10 ml	100 x 505	glycerol	0.5%
		glucose	0.05%
2 ml	1 M MgSO ₄	MgSO ₄	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

MDG non-inducing medium

This medium is used for growing working cultures (such as overnight pre-cultures) and freezer cultures (glycerol stocks).

955 ml	sterile water		
20 ml	50 x M	Na ₂ HPO ₄	25 mM
		KH ₂ PO ₄	25 mM
		NH ₄ Cl	50 mM
		Na ₂ SO ₄	5 mM
12.5 ml	40% glucose	glucose	0.5%
10 ml	25% aspartate	aspartate	0.25%
2 ml	1 M MgSO ₄	MgSO ₄	2 mM
0.2 ml	1000 x trace elements	trace elements	0.2 x

Stock solutions

ZY	1% tryptone	10 g/L
	0.5% yeast extract	5 g/L

Dissolve tryptone and yeast extract in the necessary amount of water to make up 1 L of total medium (*e.g.* 958 ml for ZYM-5052).

50 x M	1.25 M Na ₂ HPO ₄ -7H ₂ O	335 g/L
	1.25 M KH ₂ PO ₄	170 g/L
	2.5 M NH ₄ Cl	134 g/L
	0.25 M Na ₂ SO ₄	35.5 g/L

Add in sequence to 700 ml water and stir until all salts have been dissolved. Occasionally crystals appear but they can be re-dissolved in the microwave. The pH of a 20-fold dilution in water should be ~6.75.

20 x P	1.0 M Na ₂ HPO ₄ -7H ₂ O	268 g/L
	1.0 M KH ₂ PO ₄	136 g/L
	0.5 M (NH ₄) ₂ SO ₄	66 g/L

Add in sequence to 770 ml water and stir until all salts have been dissolved. The pH of a 20-fold dilution in water should be ~6.75.

100 x 505	50% glycerol	500 g/L
	5% glucose	50 g/L

Add in sequence to 570 ml water and stir until all components have been dissolved.

50 x 5052	25% glycerol	250 g/L
	2.5% glucose	25 g/L
	10% α-lactose	100 g/L

Add in sequence to 730 ml water and stir until all components have been dissolved. Lactose is slow to dissolve. It may take two hours or more at room temperature. The process can be sped up by heating in microwave.

500 x MgSO₄	1 M MgSO ₄ -7H ₂ O	24.65 g/100 ml
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For 100 ml stock solution dissolve 24.65 g MgSO₄ in 87 ml water.

Stock solutions (cont.)

80 x G	40% glucose	40 g/100 ml
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To make 100 ml stock solution add 40 g glucose to 74 ml water and stir until all glucose has been dissolved. It may take 45 min or more at room temperature. The process can be sped up by heating in microwave.

100 x D	25% aspartate	25 g/100 ml
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For 100 ml stock solution dissolve 25 g aspartic acid in 84 ml water and neutralize with 8 g NaOH (pH ~7).

1000x trace elements	50 mM FeCl ₃
	20 mM CaCl ₂
	10 mM MnCl ₂
	10 mM ZnSO ₄
	2 mM CoCl ₂
	2 mM CuCl ₂
	2 mM NiCl ₂
	2 mM Na ₂ MoO ₄
	2 mM Na ₂ SeO ₃
	2 mM H ₃ BO ₃

Preparation of 100 ml 1000x trace elements stock solution (in ~60 mM HCl).

Prepare stock solutions of the salts (except FeCl₃) as mentioned in the table below.

Dissolve 0.1 M FeCl₃ in 50 ml of 100x diluted concentrated HCl.

Add to 36 ml sterile water:

50 ml	0.1 M FeCl ₃ -6H ₂ O	2.70 g/100 ml
2 ml	1.0 M CaCl ₂ -2H ₂ O	15.8 g/100 ml
1 ml	1.0 M MnCl ₂ -4H ₂ O	19.8 g/100 ml
1 ml	1.0 M ZnSO ₄ -7H ₂ O	28.8 g/100 ml
1 ml	0.2 M CoCl ₂ -6H ₂ O	4.76 g/100 ml
2 ml	0.1 M CuCl ₂ -2H ₂ O	1.70 g/100 ml
1 ml	0.2 M NiCl ₂ -6H ₂ O	4.76 g/100 ml
2 ml	0.1 M Na ₂ MoO ₄ -2H ₂ O	2.42 g/100 ml
2 ml	0.1 M Na ₂ SeO ₃	1.73 g/100 ml
2 ml	0.1 M H ₃ BO ₃	0.62 g/100 ml

Chemicals

Compound	mol. weight	company	order number
tryptone		Sigma	T-9410
yeast extract		Sigma	Y-1000
glycerol	92.10	Roth	3783.1
glucose	180.16	Roth	X997.2
α -lactose-H ₂ O	360.32	Roth	6868.2
Na ₂ HPO ₄ -7H ₂ O	268.07	Sigma	S-9390
KH ₂ PO ₄	136.09	Sigma	P-5379
NH ₄ Cl	53.49	Merck	1145.1000
Na ₂ SO ₄	142.0	Sigma	S-6264
(NH ₄) ₂ SO ₄	132.1	OLS	2701316
MgSO ₄ -7H ₂ O	246.48	Roth	P072.2
aspartic acid	133.1	Sigma	A-8949
FeCl ₃ -6H ₂ O	270.30	Sigma	F-2877
CaCl ₂ -2H ₂ O	158.2	Sigma	C-3881
MnCl ₂ -4H ₂ O	197.91	Sigma	M-3634
ZnSO ₄ -7H ₂ O	287.56	Sigma	Z-4750
CoCl ₂ -6H ₂ O	237.95	Sigma	C-8661
CuCl ₂ -2H ₂ O	170.48	Sigma	C-6641
NiCl ₂ -6H ₂ O	237.72	Sigma	N-5756
Na ₂ MoO ₄ -2H ₂ O	241.9	Sigma	M-1003
Na ₂ SeO ₃	172.9	Sigma	S-5261
H ₃ BO ₃	61.83	Merck	165.1000