

ZYP-5052

Rich media for auto induction. Vols are ml. Add in this order!

	200 ml	500 ml	1 L	2L	3L	4L	8L
ZY media	186	464	928	1856	2784	3712	7424
1 M MgSO4	0.2	0.5	1	2	3	4	8
1000X metals mix	0.2	0.5	1	2	3	4	8
50x5052	4	10	20	40	60	80	160
20XNPS	10	25	50	100	150	200	400
antibiotics as needed							
final vol	200.4	500	1000	2000	3000	4000	8000

Sterile filter if storing

ZY media

Additional ingredients are added to ZY media, so the final volume in the aliquoted bottle is ~10% of the total

	g/ 18.6 L	g/1860 ml	g/930 ml	g/ 3720 ml
tryptone	200	20	10	40
yeast extract	100	10	5	20

Autoclave

1M MgSO4

Sterile filter

1000X metals mix - see additional word document

50x5052 - 1X = 0.5% glycerol, 0.05% glucose, 0.2% alpha-lactose

✓ glycerol by weight	25 g	250 g		125 g
H2O	73 ml	730 ml		365 ml
✓ glucose (as dextrose)	2.5 g	25 g	500 ml	12.5 g
✓ alpha-lactose	10 g	100 g		50 g
final vol	100 ml	1 L		500 ml

add in sequence, lactose is slow to dissolve, heat helps

Autoclave

20X NPS- 1X=200mM PO4, 25mM SO4, 50mM NH4, 100mM Na, 50mM K

H2O	80 ml	800 ml	1600 ml
Ammonium sulfate (NH4)2SO4	6.6 g	66 g	132 g
(Metabolic) Potassium Phosphate KH2PO4	13.6 g	136 g	272 g
Na2HPO4 (anhydrous)	14.2 g	142 g	284 g
final vol	100 ml	1 L	2L

add in sequence, pH of 1X should be ~6.75

Autoclave

Trace Metals

Lack of trace metals becomes limiting for growth in P-0.5G without added metals. Iron, manganese and cobalt were the most effective in relieving this limitation. A concentration of 0.1x trace metals mixture is sufficient to support maximal growth in P-0.5G. Growth in ZYP medium is not limited by lack of trace metals.

The 1x trace metals mixture is an attempt to saturate almost any metal-containing target protein, even at high levels of expression. The 1x concentrations are below toxic levels, as tested by growth in different concentrations of the metals individually. Target proteins produced at 100 mg/liter would have a concentration of 2 μ M for a protein of 50,000 Da or 10 μ M for a protein of 10,000 Da. If the metal content of an expressed protein is known, a saturating amount of that metal can be added rather than 1x metals mix.

1000x trace metals mixture (100 ml in ~50 mM HCl)

Add to 36 ml sterile water:

use:	of	stock solutions	MW	1x conc	to make stocks:
				(final)	g/x ml dil HCl
50	ml	0.1 M FeCl ₃ -6H ₂ O	270.30	50 M Fe	13.52g / 500ml
1/2	ml	1 M CaCl ₂	110.99	20 M Ca	11.10 g / 100ml
1	ml	1 M MnCl ₂ -4H ₂ O	197.91	10 M Mn	9.90 g / 50 ml
1	ml	1 M ZnSO ₄ -7H ₂ O	287.56	10 M Zn	14.38 g / 50 ml
1	ml	0.2 M CoCl ₂ -6H ₂ O	237.95	2 M Co	2.38 g / 50 ml
2	ml	0.1 M CuCl ₂ -2H ₂ O	170.486	2 M Cu	1.70 g / 100 ml
1	ml	0.2 M NiCl ₂ -6H ₂ O	237.72	2 M Ni	2.38 g / 50 ml
2	ml	0.1 M Na ₂ MoO ₄ -5H ₂ O	241.98	2 M Mo	2.42 g / 100 ml
2	ml	0.1 M Na ₂ SeO ₃ -5H ₂ O	263.03	2 M Se	2.63 g / 100 ml
2	ml	0.1 M H ₃ BO ₃	61.83	2 M H ₃ BO ₃	0.618 g / 100 ml

Autoclave the stock solutions of the individual metals, except 0.1 M FeCl₃ in 1/100 volume conc HCl.

A brief precipitate appeared upon addition of Na₂SeO₃, which redissolved rapidly