

Medium Preparation of Autoinduction Media

Modified from Li, et. al., 2011

1. Prepare all stock solution as in Table 1

Name	Working concentration	Stock solution preparation	Sterilization method
50 X MgSO ₄ stock solution	0.586 g L ⁻¹ MgSO ₄	Add 30 g MgSO ₄ ·7H ₂ O (MW: 246.47) to 500 ml graduated cylinder. Bring final volume to 0.5 L with water (Mill-Q or similar quality)	121°C autoclave or filtration (0.2 μm)
4 X 2.94-11.07-7.6 carbon source stock solution	2.94 g L ⁻¹ glucose 11.07 g L ⁻¹ glycerol 7.6 g L ⁻¹ lactose	Add 12.92 g glucose·H ₂ O (MW: 198.17), 44.28 g glycerol (MW: 92.09) and 32 g lactose·H ₂ O (MW: 360.31) to 1 L graduated cylinder. Bring final volume to 1 L with water (Mill-Q or similar quality)	121°C autoclave or filtration (0.2 μm)
5 X 2.94-7.38-3.8 carbon source stock solution	2.94 g L ⁻¹ glucose 7.38 g L ⁻¹ glycerol 3.8 g L ⁻¹ lactose	Add 16.15 g glucose·H ₂ O (MW: 198.17), 36.9 g glycerol (MW: 92.09) and 20 g lactose·H ₂ O (MW: 360.31) to 1 L graduated cylinder. Bring final volume to 1 L with water (Mill-Q or similar quality)	121°C autoclave or filtration (0.2 μm)
5000 X Na ₂ MoO ₄ ·H ₂ O stock solution	2.1 mg L ⁻¹ Na ₂ MoO ₄ ·2H ₂ O	Add 525 mg Na ₂ MoO ₄ ·2H ₂ O (MW: 241.95) to 50 ml graduated cylinder. Bring final volume to 50 mL with water (Mill-Q or similar quality)	Filtration (0.2 μm)
2000 X trace element stock solution	2.5 mg L ⁻¹ CoCl ₂ ·6H ₂ O 15 mg L ⁻¹ MnCl ₂ ·4H ₂ O 1.5 mg L ⁻¹ CuCl ₂ ·2H ₂ O 3 mg L ⁻¹ H ₃ BO ₃ 33.8 mg L ⁻¹ Zn(CH ₃ COO) ₂ ·2H ₂ O 14.10 mg L ⁻¹ Titriplex III	Add 0.5 g CoCl ₂ ·6H ₂ O (MW: 237.93), 3 g MnCl ₂ ·4H ₂ O (MW: 197.9), 0.3 g CuCl ₂ ·2H ₂ O (MW: 170.48), 0.6 g H ₃ BO ₃ (MW: 61.83), 6.76 g of Zn(CH ₃ COO) ₂ ·2H ₂ O (MW: 219.49), 2.82 g Titriplex III (MW: 372.24) to 100 ml graduated cylinder. Bring final volume to 100 mL with water (Mill-Q or similar quality)	Filtration (0.2 μm)
50 X (NH ₄) ₂ HPO ₄ stock solution	4 g L ⁻¹ (NH ₄) ₂ HPO ₄	Add 100 g (NH ₄) ₂ HPO ₄ (MW: 132.06) to 500 ml graduated cylinder. Bring final volume to 500 mL with water (Mill-Q or similar quality)	121°C autoclave or filtration (0.2 μm)
10 X KH ₂ PO ₄ stock solution	13.3 g L ⁻¹ KH ₂ PO ₄	Add 133 g KH ₂ PO ₄ (MW: 136.09) to 1 L graduated cylinder. Bring final volume to 1 L with water (Mill-Q or similar quality)	121°C autoclave or filtration (0.2 μm)
50 X Citric acid stock solution	1.5542 g L ⁻¹ Citric acid	Add 42.5 g Citric acid·H ₂ O (MW: 210.14) [or 38.855 g Citric acid (MW: 192.12)] to 500 ml graduated cylinder. Bring final volume to 500 mL with water (Mill-Q or similar quality)	121°C autoclave or filtration (0.2 μm)
50 X Fe(III) citrate stock solution	0.1008 g L ⁻¹ Fe(III) citrate	Add 2.52 g Fe(III) citrate (MW: 244.94) to 500 ml graduated cylinder. Bring final volume to 500 mL with water (Mill-Q or similar quality)	121°C autoclave or filtration (0.2 μm)
25 X NH ₄ OH stock solution	To bring final pH to 6.8	Add 50 ml Ammonium hydroxide (30~33%) to 500 ml graduated cylinder. Bring final volume to 500 mL with water (Mill-Q or similar quality)	

Table 1. Preparation of stock solutions

All stock solutions are autoclaved prior to mixing unless filtration is required. Each stock solutions is contained in a different bottle.

2. Mixing

Mixing is done by adding each of stock solution in order as listed in Table 2 without making Group A and Group B solutions. Mixing is done under aseptic condition in Biosafety Cabinet. Once the autoinduction media has been made, store at 4°C or keep refrigerated.

Groups		Name of medium	S-DAB (HNC)
		Application of the medium	Good oxygen supply
		Inducer	Lactose
		Volume of stock solution	ml
A group	Magnesium*	50 X MgSO ₄ stock solution	20
	Carbon source*	4 X 2.94-11.07-7.6 carbon source stock solution	250
		5 X 2.94-7.38-3.8 carbon source stock solution	
		20 X Glucose stock solution	
	Trace**	5000 times Na ₂ MoO ₄ H ₂ O stock solution	0.2
B group	Elements**	2000 times trace element stock solution	0.5
	Nitrogen*	50 X (NH ₄) ₂ HPO ₄ stock solution	20
	Phosphate and other salts*	10 X KH ₂ PO ₄ stock solution	100
		50 X Citric acid stock solution	20
		50 X Fe(III) citrate stock solution	20
	pH adjusting*	25 X NH ₄ OH stock solution	45~50
		50 X 5 mol L ⁻¹ NaOH stock solution	
Solvent*	Water (Milli-Q or similar quality)	520	

Table 2. Medium preparation

References:

Li, Z., Kessler, W., Van Den Heuvel, J., Rinas, U. Simple defined autoinduction medium for high-level recombinant protein production using T7-based *Escherichia coli* expression systems. (2011) *Applied Microbiology and Biotechnology*, 91 (4), pp. 1203-1213.