E.coli Expression and Renaturing Protocols

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1. High efficiency competent *E.coli* cells

(i) Grow bugs in LB or similar - 500 ml for good batch

(ii) When grown to OD 600 of 0.6, spin down gently.

(iii) Resuspend in 100mls cold TFB1 and then spin down again

(iv) Resuspend in 20mls cold TFB2 - aliquot and store at -80^{o}C.

TFB1

30mM KOAc
50mM MnCl2
100mM KCl
10mM CaCl2
15% v/v glycerol

TFB2

10mM Na-MOPS pH 7.0
75 mM CaCl2
10mM KCl
15% v/v glycerol

2. Expression media

	Low salt LB, 1 litre	**Normal LB, l litre**	**TYP, 1 litre**
Bactotryptone	10g	10g	16g
Yeast extract	5g	5g	16g
NaCl	5g	10g	5g
K2HPO4 (anhydrous)	-	-	2.5g

3. Inclusion body preparation

Triton Wash

50 mM Tris-HCl pH 8.0	50mls 1M
0.5% Triton-X100	5mls 100%
200 mM NaCl	40mls 5M
10 mM EDTA	20mls 0.5M
0.1 % (w/v) Sodium Azide	10mls 10%
2 mM DTT, pH 8.0	1ml 2M (or 100ul/100mls)

Make up to 1 litre with water

Resuspension wash

50 mM Tris-HCl pH 8.0	50mls 1M
1 mM EDTA	2mls 0.5M
0.1 % (w/v) Sodium Azide	10mls 10%
2 mM DTT	1ml 2M (or 100ul/100mls)

Make up to 1 litre with water

4. Inclusion body solubilisation

Urea solution

8M Urea	40 mls 10M urea
50mM MES pH 6.5	2.5 mls 1M Mes pH 6.5
10mM EDTA	1 ml 0.5M EDTA
2mM DTT	50ul 2M DTT
H2O	6.45 mls
	total: 50 mls

Guanidine solution

6M GdmHCL	15mls 8M Gdm
50mM Mes pH 6.5	1ml of 1M Mes pH 6.5
10mM EDTA	400ml of 0.5M EDTA
2mM DTT	20ml of 2M DTT
H2O	3.58 mls
	total: 20mls

4. Refolding buffer

100mM Tris pH 8.0	100mls 1M
2mM EDTA	4mls 0.5M
0.4M L-Arginine-Hydrochloride	84.28g
5mM Oxidised Glutathione	0.31g
0.5mM Reduced Glutathione	1.54g
0.1mM PMSF	1 ml 0.1M

Make up to 1litre with water

5. FPLC buffer

150mM NaCl	30mls 5M
20mM Tris pH 8.0	20mls 1M

Make up to 1 litre with water