BUFFERS AND REAGENTS

Part A: For S-30 prep

1. Growth media (4L)
    - 22.4g KH_2PO_4
    - 115.6g K_2HPO_4
    - 40g yeast extract

    Divide into 3 1L flasks, 5-100ml flasks, and 5-100ml bottles, autoclave. Prior to inoculation, add 40ml of 25% filtered glucose per litre of media.
2. Wash buffer (1L)
    - 10mM Tris-acetate, pH 8.0
    - 14mM $Mg(acetate)_2$
    - 60mM KCl
    - 6mM B-mercaptoethanol (just prior to use)
    - 50(g/mL PMSF (just prior to use)
3. Lysis buffer (100ml)
    - 10mM Tris-acetate, pH 8.0
    - 14mM $Mg(acetate)_2$
    - 60mM KCl
    - 50(g/mL PMSF (just prior to use)
4. Preincubation mix (10ml)
    - 3.75ml of 2M Tris-Ac, ph 8.0 (make up fresh)
    - 213uL of 1M $Mg(acetate)_2$
    - 750uL of 19 aa's - met (1mM each)
    - 37.5uL of 20mM methionine
    - 600uL of 100mM ATP
    - 200mg of phosphoenol pyruvate (PEP)
    - 50 units pyruvate kinase
    - 75uL of 1M DTT
    - H_2O to 10 mL
5. Dialysis buffer (8L)
    - 10mM Tris-Acetate, pH 8.0
    - 14mM $Mg(acetate)_2$
    - 60mM K(acetate)
    - 1mM DTT (just prior to use)

Part B: for mfrs prep:

1. TB (2L)
    - 24g bactotryptone
    - 48g yeast extract
    - 8ml glycerol

    Autoclave, allow to cool to < 60(C, then add 200ml of sterile, filtered 0.17M KH2PO4, 0.72M K2HPO4.
2. Buffer A (100ml)
    - 10mM Tris -Acetate, pH 8.0
    - 14mM Mg (acetate)2
    - 60mM K (acetate)
    - 0.1mM DTT (just prior to use)
    - 0.5mM PMSF (just prior to use)
3. Buffer B (200 mL)
    - 10mM Tris-Acetate, pH 8.0
    - 14mM Mg (acetate)2
    - 60mM K (acetate)
    - 0.5% Triton X - 100
    - 0.1mM DTT (just prior to use)
4. Buffer C (100ml)
    - 10mM Tris- Acetate, pH 8.0
    - 14mM Mg (acetate)2
    - 60mM K (acetate)
    - 0.1mM DTT (just prior to use)

Part C: 2.5 X reaction mixture:

FINAL	STOCK	VOLUME/4mL of 2.5X (uL)
35mM Tris-Acetate pH 8.0	2M	175
190mM K(glutamate)	2M	950
30mM NH4OAc	5M	60
2mM DTT	1M	20
12mM Mg(OAc)2	1M	120
40uM 19aa's- met	1M	400

2mM ATP	100mM	200
0.5mM each of CTP, UTP, GTP	15mM	330
20mM phosphoenol pyruvate	400mM	500
0.1mg/mL E.coli tRNA	10mg/mL	100
35mg/mL PEG 8000	400mg/mL	875
20 ug/mL folinic acid	4000ug/mL	50
2mM IPTG	100mM	200
H2O to 4mL		