

Buffer and Media Preparation

Aims of the experiment

Buffer is used for the cell extract production including cell washing, diluting and dialysis. Media is essential to provide a platform for bacteria to grow efficiently. They are prepared according Sun, Z. Z et al., 2013. For each cell extract production in shaking flasks, it requires approximately 1.5 liters 2YT+P media, 1.25 liters S30A and 1.5 liters S30B buffer for 3ml final cell extract.

Materials

- 2xYT – Medium
- Potassium phosphate dibasic (K_2HPO_4)
- Potassium phosphate monobasic (KH_2PO_4)
- Mg-glutamate
- K-glutamate
- Tris
- 2M Tris base
- acetic acid
- 1M DTT

Procedure

1. Prepare 2YT+P Media according to the following table. Amount depends on the amount of cells cultivated according to different cultivation methods.

Concentration, mol/l (M)	Reagents
0.022	KH_2PO_4
0.040	K_2HPO_4
31g/L	2xYT

2. Store media at room temperature after autoclaving.

3. Prepare S30A buffer according to the following table.

Concentration, mol/l (M)	Reagents
0.014	Mg-Glutamate
0.060	K-Glutamate
0.050	Tris
2	DTT (add just before use)
Titrate until pH 7.7	acetic acid

4. Prepare S30B buffer according to the following table.

Concentration, mol/l (M)	Reagents
0.014	Mg-Glutamate
0.060	K-Glutamate
1	DTT (add just before use)
Titrate until pH 8.2	2M Tris base

5. Store both buffers at 4°C after autoclaving.