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Preparation of a cell-free expression system from Escherichia coli

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Protocol status: Working
This protocol is based on a published paper, it has been tried and tested under different conditions. All these have been mentioned. Please feel free to get in touch with any comments.

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Abstract

This protocol describes the procedure make an S30 derived E.coli cell lysate that lacks membrane components. Modified from a protocol published in https://doi.org/10.1091/mbc.e11-07-0590
The procedure takes three days in total, including preparation time.

Guidelines

Start up ultra-centrifuges 30 min prior to use to stabilise vacuum. Work on ice. Plot growth curves for the strain used in the medium used for preparation (see materials.)

Materials

MATERIALS

- Magnesium acetate
- Sodium Hydroxide **Fisher Scientific** **Catalog #**BP359500
- L-aminoacids **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**LAA21–1KT
- DTT **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D0632
- NaCl **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**53014
- Tris acetate **Bio Basic Inc.** **Catalog #**TD0101.SIZE.500g
- BD Bacto™ Yeast Extract **Becton Dickinson (BD)** **Catalog #**212750
- Sucrose **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S7903
- Potassium acetate **Merck Millipore (EMD Millipore)** **Catalog #**1.04820.1000
- Tryptone (pancreatic digest of casein) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T9410
- Phospho(enol)pyruvic acid mono potassium salt **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**860077
- Adenosine 5′-triphosphate (ATP) disodium salt hydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A2383
- Pyruvate kinase from rabbit muscle **Catalog #**83330

Cells can be cultured in LB medium (buffered with phosphates), TB or S30 medium. Prepare 4 litres i.e. 1 litre each in 4 5 litre erlenmeyer flasks. Autoclave.

S30 medium
9 g/l bacto-tryptone
0.8 g/l yeast extract
5.6 g/l NaCl
1 mM NaOH

Prepare a mix of the 20 amino acids 1 mM each. Aliquot and store at -20°C.
Prepare stock solutions of the acetates: 1M Tris Acetate at pH 7.5, 1M Magnesium acetate at pH 7.5, 4 M Potassium acetate at pH 7.5
Prepare stock solutions: 0.25 M ATP pH neutralised with KOH, 0.2 M PEP. Make aliquots and store at -20°C.

Strains tested: E.coli KC6, MRE600, MC4100.

If using a rich medium, do a growth curve for the strain being used under those growth conditions, and harvest at early exponential phase.

S30 buffer (pH 7.5)

10 mM Tris-acetate
14 mM magnesium acetate
60 mM potassium acetate
1 mM DTT

Sucrose cushion (pH 7.5)
10 mM Tris acetate
14 mM Magnesium acetate
60 mM potassium acetate
1 mM DTT
1.44 M sucrose

Safety warnings

Use ultracentrifuges carefully, ensure to fill tubes up to requisite volumes. Handle chemicals according to local safety requirements. Wear eye-goggles and a mask while weighing yeast extract and peptone. Use fume hoods where indicated.
Handle sharps with care. Dispose off responsibly.
Handle liquid nitrogen with cryo-protective gear including eye goggles.
Handle autoclaves carefully.

Before start

Wash all flasks and bottles with milliQ water. Prepare all buffers in milliQ water.