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Cell growth and harvest for E. coli-based Cell-free Protein Synthesis

In 1 collection

Weston Kightlinger[1]

[1]SwiftScale Biologics, Inc

1 Works for me Share dx.doi.org/10.17504/protocols.io.bvh4n38w

SwiftScale Biologics

E Emily Pennell

ABSTRACT

Cell-free protein synthesis reactions require cell-free extract derived from rapidly dividing E. coli cells to produce high yields of protein therapeutics, antigens, enzymes, vaccines, and other proteins. This protocol describes the cell growth and harvest steps for E. coli to produce extract.

Read more on cell-free protein synthesis here: https://www.swiftscalebio.com/blog/cell-free-protein-synthesis

ATTACHMENTS

dn6kbkfmx.pdf

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PROTOCOL CITATION

Weston Kightlinger 2021. Cell growth and harvest for E. coli-based Cell-free Protein Synthesis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvh4n38w

COLLECTIONS

Collection of protocols for cell-free protein synthesis

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PARENT PROTOCOLS

Part of collection

Collection of protocols for cell-free protein synthesis

MATERIALS TEXT

Materials:

1. **2.5 L** Tunair flask with baffles
2. Autoclavable **250 mL** glass bottles
3. Media components (see below)

A	B
2xYTPG Total Volume (mL) =	1000
2xYTP*** Total Volume (mL) =	750
NaCl (g)	5
Tryptone (g)	16
Yeast Extract (g)	10
Potassium Phosphate, dibasic (K2HPO4) (g)	7
Potassium Phosphate, monobasic (KH2PO4) g)	3
Glucose Total Volume (mL) =	250
Glucose (g)	18

***Adjust 2XYTP pH to 7.2 using 5 N KOH.

4. MilliQ water
5. **2.5 L** Tunair flask with baffles
6. Chosen E. coli strain (often BL21 (DE3) in standard protocols)
7. **1 L** 2xYTPG (**750 mL** of 2xYTP media in Tunair flask and autoclaved, **250 mL** of **72 g/L** glucose in a **250 mL** glass bottle and autoclaved)
8. LB Media
9. S30 Buffer Components:

a. 100X: **1 Molarity (M)** Tris-Acetate **pH 8.2** from Trizma Base, adjusted with Glacial Acetic Acid.

b. 100X: **1.4 Molarity (M)** Mg(Acetate)2 from Mg(OAc)2 Tetrahydrate

c. 100X: **6.0 Molarity (M)** K(Acetate) **588.90 g** K(OAc)

d. Optionally, DTT can be added at **2 Milimolar (mM)** using **1 Molarity (M)** DTT as a 500X stock.

Cell growth and harvest for E. coli-based Cell-free Protein Synthesis 5m

1

Start a **50 mL** seed culture of E. coli strain and grow in LB media **Overnight** at **37 °C** .

2

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Add **250 mL** glucose to **750 mL** 2xYTP media in Tunair flask to obtain 2xYTPG media.

3 Inoculate Tunair flask culture at approximately OD_{600} = 0.05.

4 

Grow culture at **37 °C** with shaking at **250 rpm** to OD_{600} = 0.6 and induce T7 with IPTG (if necessary). Add to **1 Milimolar (mM)** IPTG.

5 

5m

Harvest culture at OD_{600} = 3 by centrifugation at **8000 x g** for **00:05:00** and washing with S30 buffer three times.

6 After the last wash, pour off S30 supernatant to obtain the pellet. Resuspend this pellet in S30 and lyse or immediately flash freeze on liquid nitrogen and store at **-80 °C** until ready for cell lysis.

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