



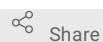
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Media Preparation for E. coli growth for Cell-free Protein Synthesis

In 1 collection

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1 Works for me



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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. Standard protocols use 2xYTPG media for cell growth. This protocol is for making 1 L of 2xYTPG media.

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

ATTACHMENTS

[dn6kbkfm.pdf](#)

DOI

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PROTOCOL CITATION

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COLLECTIONS ⓘ

Collection of protocols for cell-free protein synthesis

KEYWORDS

E. coli growth, Cell-free Protein Synthesis, Media Preparation

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50459



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)
4. MilliQ water

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 (K ₂ HPO ₄) (g)	7
Potassium Phosphate, monobasic (KH ₂ PO ₄) (g)	3
Glucose Total Volume (mL) =	250
Glucose (g)	18

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

Media Preparation for E. coli growth for Cell-free Protein Synthesis1 

Mix ingredients except for glucose with water below the amount of volume of MilliQ water needed using a plastic beaker and a stir-bar. Once dissolved (you can use a spatula to help break up clumps), pour into a big plastic graduated cylinder and adjust to final volume then mix in original beaker then disperse  **750 mL** to  **2.5 L** Tunair flasks for autoclaving.

2 Prepare the glucose separately in individual  **250 mL** containers and add just before inoculation.

3 Calibrate pH probe.

4 pH the 2xYTP solution to **pH7.2** using 5 N KOH.

Expected Result: Expected concentration of protein in Cell-free extract is ~ **[M]40 mg/ml** so make the following dilutions: