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# Protein expression of hard-to-produce proteins in the cytoplasm of *Escherichia coli*

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1 Works for me [dx.doi.org/10.17504/protocols.io.bfbwjipe](https://dx.doi.org/10.17504/protocols.io.bfbwjipe)

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## ABSTRACT

This protocol describes the expression and extraction of heterologously cytoplasmically expressed proteins in the bacterium *E. coli* for T7 based expression systems.

Specifically, it describes how to produce "hard-to-produce", as for example, LPMOs.

It is based on a modified version of:

"IPTG Induction and Extraction of Proteins from Bacteria" by Swathi Arur and Sudhir Nayak, Schedl Lab.

Washington University Genetics, St. Louis.

Hemsworth, G. R., Henrissat, B., Davies, G. J., and Walton, P. H. (2014) Discovery and characterization of a new family of lytic polysaccharide monooxygenases. Nat. Chem. Biol. 10, 122–126.

The extraction of cytoplasmatic proteins is followed by a chemical lysis protocol, alternatively, sonication can also be employed.

## DOI

[dx.doi.org/10.17504/protocols.io.bfbwjipe](https://dx.doi.org/10.17504/protocols.io.bfbwjipe)

## PROTOCOL CITATION

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**protocols.io**

<https://dx.doi.org/10.17504/protocols.io.bfbwjipe>

## KEYWORDS

*E. coli*, T7, Protein production, Cytoplasmic expression, sHuffle strain, disulfide bond

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## CREATED

Apr 20, 2020

## LAST MODIFIED

Oct 27, 2020

## PROTOCOL INTEGER ID

35926

## GUIDELINES

Make sure to clone your favorite gene for expression in the appropriate expression vector.

#### MATERIALS TEXT

#### MATERIALS

☒ [SHuffle T7 Competent E.coli - 6x0.05 ml](#) **New England**

**Biolabs Catalog #C3026H**

☒ [IPTG Bio Basic](#)

**Inc. Catalog #IB0168.SIZE.100g**

☒ [BL21\(DE3\) or BL21-Star\(DE3\) or Rosetta2\(DE3\) or etc for protein purification](#) **Contributed by users**

☒ [Kanamycin Research Products International](#)

**(rpi) Catalog #K22000-25.0**

☒ [LB Research Products International](#)

**(rpi) Catalog #L24400-2000.0**

#### SAFETY WARNINGS

This protocol describes the construction of GMO classified organisms. Make sure that the local GMO and safety legislations are respected.

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Specifically, it describes how to produce "hard-to-produce", as for example, LPMOs.

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#### BEFORE STARTING

Prepare a fresh transformation of your expression vector in *E. coli*/BL21 (DE3) cells or other appropriate strain (e.g. sHuffle strain, Rosetta, etc.)

#### Day 1 - Culture inoculation

- 1 Pick a fresh colony of your BL21 (DE3) strain previously transformed with your expression vector, and inoculate it in LB medium supplemented with relevant antibiotics. Grow the culture at  **37 °C** at 250 RPM shaking  **Overnight** .  
The volume of the overnight culture depends on the volume of the expression culture and should be at least 1/100 of the expression culture

#### Day 2 - Induction

- 2 Dilute the overnight culture 1:100 in fresh LB supplemented with relevant antibiotics
- 3 Grow the culture at  **37 °C** with 250 RPM shaking until an OD<sub>600</sub> = 0.5 - 0.6

- 4 Move the culture into an incubator set to **18 °C** with 180 RPM of shaking and allow the culture to reach  $OD_{600} = 0.8 - 1.0$
- 5 Induce the expression by adding IPTG to a final concentration of **1 Milimolar (mM)**
- 6 Grow the culture for **20:00:00** at **18 °C** with 180 RPM shaking.



The temperature can be lowered further allowing for a slower induction in order to increase the production of soluble product.

#### Day 3 - Collection and lysis

- 7 Collect induced samples by centrifugation at **8000 x g, 4°C, 00:20:00** and carefully discard the supernatant



Pellets can be frozen here at **-80 °C** or **-20 °C** until protein extraction

- 8 Lyse the cells using a lysis protocol or a commercially available kit
- 9 Keep the extractions **On ice** when working with it and at **4 °C** for storage