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## Protein expression in OnePot PURE cell-free system [↗](#)

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Works for me

[dx.doi.org/10.17504/protocols.io.8avhse6](https://doi.org/10.17504/protocols.io.8avhse6)

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### ABSTRACT

In this protocol we explain the procedure of protein expression in the OnePot PURE cell-free system.

### EXTERNAL LINK

<https://pubs.acs.org/doi/10.1021/acssynbio.8b00427>

### MATERIALS TEXT

Material/Consumables:

- OnePot Protein solution
- OnePot Ribosome solution
- OnePot Energy solution
- DNA template
- Nuclease free water

| Component         | Volume (μL) | Final concentration of the reaction (nM) |
|-------------------|-------------|--|
| Protein Solution  | 0.65        | -  |
| Ribosome Solution | 0.9         | -  |
| Energy Solution   | 2           | -  |
| DNA Template      | x           | 5  |
| Water             | 1.45-x      | -  |



### BEFORE STARTING

In order to create the components needed refer to the following protocols:

**Protein Purification for OnePot PURE cell-free system**  
by Konstantinos Ragios

PREVIEW



RUN



Ribosome Purification for OnePot PURE cell-free system  
by Konstantinos Ragios

PREVIEW

RUN



Energy solution preparation for OnePot PURE cell-free system  
by Konstantinos Ragios

PREVIEW

RUN

## Protein expression

- 1 For a 5µl reaction add to a tube 2µl of Energy solution, 0.65µl of Protein solution, 0.9µl of Ribosome solution, the DNA template (5nM final concentration in the reaction) and if needed add water to reach the final volume.



The minimum reaction volume is 5µl while the suggested one is 10µl.  
For any final reaction volume (e.g. X µl) you just need to multiply the volumes of the components needed by a factor of X/5.



If you haven't produce ribosomes then any store-bought product can be used.

- 2 Centrifuge for a few seconds and then your solution will start reacting.

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If you want to measure it with a plate reader, ideally you should add each component in different corners of the well, so the reaction will not initiate before adding the plate on the reader. The centrifuge for a few seconds.



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