

Oct 20, 2019

# Protein expression in OnePot PURE cell-free system

DOI

dx.doi.org/10.17504/protocols.io.8avhse6



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Protocol Citation: Konstantinos Ragios 2019. Protein expression in OnePot PURE cell-free system. protocols.io

https://dx.doi.org/10.17504/protocols.io.8avhse6



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Protocol status: Working

We use this protocol and it's working

Created: October 16, 2019

Last Modified: October 20, 2019

**Protocol Integer ID: 28725** 

**Keywords:** iGEM, cell-free, OnePot, PURE, protein, expression, protein expression in onepot pure cell, onepot pure cell, protein expression, procedure of protein expression, pure cell, free system in this protocol, cell, free system

### **Abstract**

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



## **Materials**

Material/Consumables:

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

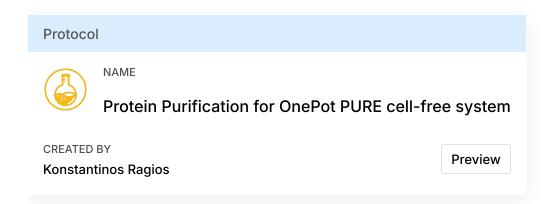
| Com<br>pon<br>ent                | Volu<br>me<br>(μL) | Fina I con cent ratio n of the reac tion (nM ) |
|----------------------------------|--------------------|--|
| Prot<br>ein<br>Solu<br>tion      | 0.65               | -  |
| Ribo<br>som<br>e<br>Solu<br>tion | 0.9                | -  |
| Ener<br>gy<br>Solu<br>tion       | 2                  | -  |
| DNA<br>Tem<br>plate              | х                  | 5  |
| Wat<br>er                        | 1.45<br>-x         | -  |

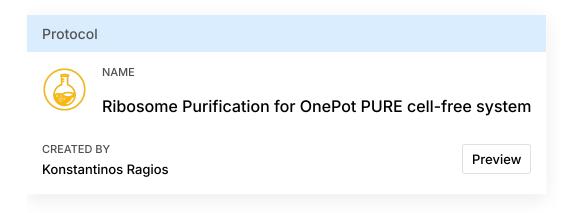
# Troubleshooting

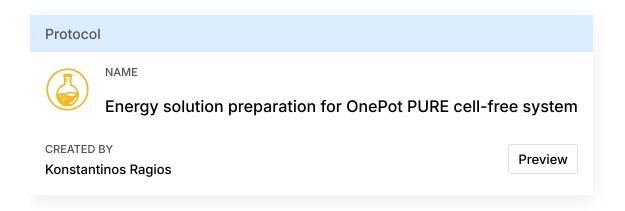


## Before start

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









## Protein expression

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.

#### Note

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

#### Note

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.

3

### Note

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.