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🌐 Protein expression in OnePot PURE cell-free system

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Protocol status: Working
We use this protocol and it's working

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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

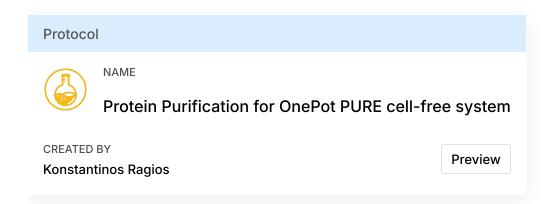| Component	Volume (μL)	Final concentration of the reaction (nM)
Protein Solution	0.65	-
Ribosome Solution	0.9	-
Energy Solution	2	-
DNA Template	x	5
Water	1.45 -x	-

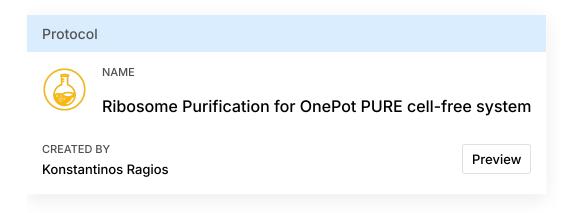# Troubleshooting

Before start

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

Protocol

NAME

Protein Purification for OnePot PURE cell-free system

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Preview

Protocol

NAME

Ribosome Purification for OnePot PURE cell-free system

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Preview

Protocol

NAME

Energy solution preparation for OnePot PURE cell-free system

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Preview

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.

> **Note**
>
> The minimum reaction volume is 5μl while the suggested one in 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.

> **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.