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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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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	-

Before start

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

Protocol

NAME

Protein Purification for OnePot PURE cell-free system

CREATED BY

Konstantinos Ragios

PREVIEW

Protocol

NAME

Ribosome Purification for OnePot PURE cell-free system

CREATED BY

Konstantinos Ragios

PREVIEW

Protocol

NAME

Energy solution preparation for OnePot PURE cell-free system

CREATED BY

Konstantinos Ragios

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.