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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

	<b>Com pon ent</b>	<b>Volu me (<math>\mu</math>L)</b>	<b>Fina l con cent ratio n of the reac tion (nM )</b>
	Prot ein Solu tion	0.65	-
	Ribo som e Solu tion	0.9	-
	Ener gy Solu tion	2	-
	DNA Tem plate	x	5
	Wat er	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 5 $\mu$ l reaction add to a tube 2 $\mu$ l of Energy solution, 0.65 $\mu$ l of Protein solution, 0.9 $\mu$ l 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 5 $\mu$ l while the suggested one is 10 $\mu$ l.  
For any final reaction volume (e.g. X  $\mu$ l) 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.