OCT 02, 2019

CDO expression into OnePot PURE

Forked from [CDO expression into OnePot PURE](#)

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OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.7v3hn8n

Protocol Citation: Dana Mozaffari 2019. CDO expression into OnePot PURE. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.7v3hn8n>

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Protocol status: In development
We are still developing and optimizing this protocol

Created: Oct 02, 2019

Last Modified: Oct 02, 2019

PROTOCOL integer ID:
28315

Keywords: OnePot PURE, CDO, Catechol, Cell Free

ABSTRACT

This protocol explains the procedure for expressing the catechol degrading enzyme *Catechol-2,3-deoxygenase* (CDO) into a "homemade" OnePot PURE cell-free transcription/translation system.

Once expressed, this enzyme degrades catechol, a colorless substrate, into a yellow colored one, 2-hydroxymuconate semialdehyde (2-HMS), providing a colorimetric signal that can be easily implemented in another protocol.

GUIDELINES

We used a CDO plasmid with A T7 promoter added to it.

MATERIALS

	Materials μ l	CDO expression (1)	Control without plasmid (2)	Control without catechol (3)
	Energy solution	2	2	2
	Ribosomes	0.9	0.9	0.9
	Proteins	0.65	0.65	0.65
	CDO Plasmid	25 ng	-	25 ng
	Catechol 10mM	0.5	0.5	-
	Water	up to 5 μ l	up to 5 μ l	up to 5 μ l
	Total	5	5	5

BEFORE START INSTRUCTIONS

Preheat the incubator at 37°C

- 1 Label 3 PCR tubes according to the reactions
- 2 In each tubes add the Energy solution, proteins and ribosomes.
- 3 Add the catechol, CDO and water as needed according to the materials chart.

- 4 Make a quick spin in the centrifuge to have all the liquid in the bottom.
- 5 Incubate one hour at 37°C