



Oct 02, 2019

CDO expression into OnePot PURE

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In Development

[dx.doi.org/10.17504/protocols.io.7nhhmb6](https://doi.org/10.17504/protocols.io.7nhhmb6)

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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 TEXT

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 STARTING

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



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