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## CDO expression into OnePot PURE

Forked from [CDO expression into OnePot PURE](#)Dana Mozaffari<sup>1</sup><sup>1</sup>EPFL - EPF Lausanne

In Development

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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 $\mu$ 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 $\mu$ l	up to 5 $\mu$ l	up to 5 $\mu$ l
<b>Total</b>	<b>5</b>	<b>5</b>	<b>5</b>

### 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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