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CDO expression into OnePot PURE



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Protocol status: In development

We are still developing and optimizing this protocol

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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

	Material s μ l	CDO expression (1)	Control with out plasmid (2)	Control with out 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

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