Oct 02, 2019

CDO expression into OnePot PURE

DOI
dx.doi.org/10.17504/protocols.io.7nhhmb6

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Protocol Citation: Laura Kvedarauskaite 2019. CDO expression into OnePot PURE. **protocols.io https://dx.doi.org/10.17504/protocols.io.7nhhmb6**

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

Created: September 25, 2019

Last Modified: October 02, 2019

Protocol Integer ID: 28073

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 semialdyhyde (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 µl	**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 µl	up to 5 µl	up to 5 µl
	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