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CFE Expression and Lyophilization of β-Galactosidase (LacZ)

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We use this protocol and it's working

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Abstract

This protocol includes the expression and lyophilization of the β-Galactosidase enzyme (from the LacZ gene) in a cell-free system. We used it to prepare and conduct experiments for a biochemistry lab course.

The cell-free extracts and buffers were prepared according to:
Guzman-Chavez, F., Arce, A., Adhikari, A., Vadhin, S., Pedroza-Garcia, J. A., Gandini, C., Ajioka, J. W., Molloy, J., Sanchez-Nieto, S., Varner, J. D., Federici, F., & Haseloff, J. (2022). Constructing Cell-Free Expression Systems for Low-Cost Access.*ACS synthetic biology*,*11*(3), 1114–1128. https://doi.org/10.1021/acssynbio.1c00342

The lyophilization steps for cell-free systems were adapted from:
Jung, J. K., Alam, K. K., Verosloff, M. S., Capdevila, D. A., Desmau, M., Clauer, P. R., ... & Lucks, J. B. (2020). Cell-free biosensors for rapid detection of water contaminants.*Nature* Biotechnology,*38*(12), 1451-1459.

Troubleshooting

CFE Expression and Lyophilization of β-Galactosidase

1

Note

For this protocol, it is considered that you **already have DNA capable of expressing β-Galactosidase at a concentration of at least 200 ng/uL**. In our case, we use **this plasmid**.

For the **cell-free expression** of β-Galactosidase, we prepare the following reaction according to **the guidelines of this article:**

	A	B	C
		1x (uL)	**25x (uL)**
	Cell-Free Extract	4	100
	Buffer PEP	3	75
	DNA (LacZ 200 ng/uL)	2	50
	H20	1	25
		10	**250**

Expected result

Here, we prepare **two additional** 10 µL reactions, one with and one without DNA, that will work as **positive and negative controls** of the CFE expression, respectively.

To each one of them, we add 1 µL of a [M] 15 mg/mL solution of Chlorophenol Red-β-D-galactopyranoside **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**59767 .

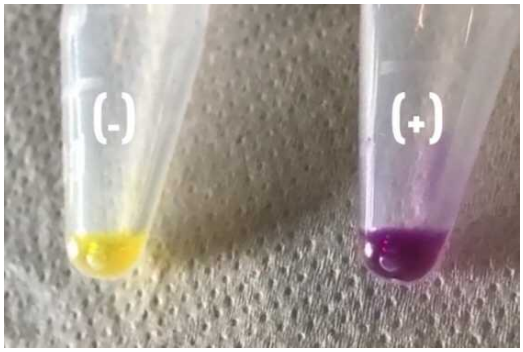
2 Vortex the tubes. Incubate Overnight at 230 rpm, 37°C in an orbital shaker. 16h

Note

We have seen that shaking improves the reaction efficiency. To ensure optimal stir, we put the 0,6 or 1,5 mL tubes in an Erlenmeyer flask, cover the flask with aluminium foil and put it in the orbital shaker.

Expected result

After incubation, the **control tubes** should look similar to this:



The tubes should have a yellow color that will only turn to purple in the case of the positive control, as the beta-galactosidase catalyzes a reaction that cleaves CPRG into galactose and chlorophenol red.

3 Prepare the CFE reactions for lyophilization by diluting 200 µL of the reaction in **PBS 1X** to a final volume of 2500 µL (1/12.5 dilution).

4 Vortex and make 50 µL aliquots. **Seal each tube** with aluminum foil or parafilm.

Note

*This should take approximately **50 tubes.** The sealing is done to prevent the small pellets from "jumping" out of the tubes under vacuum.*

5 Place the tubes at 80 °C **for 20-30 min** to allow slow freezing.

6 Perform small punctures in the seal of each tube using a small 1mL syringe or a similar instrument.

7 Transfer the tubes to a freeze-dryer and lyophilize the tubes for at least 02:00:00 . 2h

Note

In our case, our freeze-dryer allows us to set a condenser temperature of -84 °C and 0,04 mBar of pressure.

We place the tubes at this setting for 60 mins, then turn OFF the vacuum pump and let the vacuum and temperature slowly rise for 60 minutes before removing the tubes from the drying chamber

Equipment

FreeZone® 2.5 L Benchtop Freeze Dryers	NAME
Labconco®	BRAND
700202000	SKU

8 Remove the tubes from the drying chamber, **quickly remove the parafilm and close the tubes**.

Safety information

It is very important that this step is carried out quickly and with gloves, to avoid any cross-contamination that may affect subsequent steps

9 Unless rehydrated immediately, place the tubes in a light protective bag with a silica gel desiccant, an oxygen absorber and a shot of argon using an argon canister to achieve an anoxic environment. **Vacuum seal** the bag using a vacuum sealer.

10 Store the bags protected from light at 4 °C or RT

Rehydration of β-Galactosidase 40s

11 To rehydrate the lyophilized enzyme (seen as a white pellet on the tube), spin down and add 1 mL **of PBS 1X**. Vortex for 00:00:40 to favor the homogeneous resuspension of the protein inside the tube. 40s

Note

This should make a final 1/250 dilution from the original CFE reaction expressing β-Galactosidase, bringing the solution to a concentration we can use in these experiments.

To determine the ideal concentration of enzyme necessary for our experiments, we realized an enzyme assay with different concentrations of β-Galactosidase (and a constant concentration of **CPRG**). We chose the highest enzyme dilution that showed a colorimetric change from yellow to red/purple after incubating the tubes at 37°C for 15 minutes.