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β-Galactosidase Activity Assay

You will need to determine the optimal cell culture conditions for your β-Gal assay. Typically, overnight cultures are diluted 1:50 – 1:100, and then grown to mid-log or early stationary phase. (Overnight cultures can also be used directly for β-Gal assays in some cases.)

Z buffer

To make 500 mL:	Final concentrations:
150 mL 0.2 M Na_2HPO_4	0.06 M Na_2HPO_4
100 mL 0.2 M NaH_2PO_4	0.04 M NaH_2PO_4
5 mL 1 M KCl	0.01 M KCl
5 mL 0.1 M $MgSO_4$	0.001 M $MgSO_4$

pH should be 7.0; filter sterilize

Just before using, add β -mercaptoethanol (IN THE HOOD) (35 µL per 10 mL Z buffer).

1. Determine the A_{600} of the cultures
2. In a 2 mL tube, **mix** 0.1 mL cell culture with 0.9 mL Z buffer, 100 µL chloroform and 50 µL 0.1% SDS. **Vortex** the solution for 10 sec, then let the tubes **rest** at RT.
3. **Add** 0.2 mL of *o*-nitrophenyl- β -D-galactopyranoside (ONPG) in Z buffer (4 mg/mL) to the lysed culture prepared in step 2. When a yellow color develops, **stop** the reaction by adding 0.3 mL 1 M Na_2CO_3. **Record** the time at which the yellow color stops.
4. Briefly **spin** down the tubes (~1 min full speed; or even 10 seconds in picofuge)
5. Being careful to only take the aqueous (top) phase*, **remove** 1 mL of the assay solution from the tube and **transfer** to a cuvette. **Read** the A_{550} and A_{420} of the reaction samples. Units of β -galactosidase activity =

$$1000 \text{ x } \frac{A_{420} - (1.75 \text{ x } A_{550})}{t \text{ x } 0.1 \text{ x } A_{600}}$$

where t = time in minutes.

*The chloroform will mess up the plastic cuvettes

Optional:

Use PopCulture to lyse cells (EMD Biosciences)

Do in 96 well with 140 µL Z buffer, 10 µL cell lysate and 10 µL ONPG. Measure A_{420} for 20-40 minutes at 30-second intervals.