β-GALACTOSIDASE (*LACZ*) ASSAY

1. Thaw samples on ice, then prepare lysates (see notes below on sample collection and preparation of lysates)
2. Prepare several (at least 3 per sample along with a single blank) plastic cuvettes, each containing 500 µl sodium carbonate stop solution; you may wish to label these with some notation of the sample to be tested and the timepoint to be used
3. Place into a microcentrifuge tube the following:
    - 400 µl sodium phosphate buffer (pH 7.5)
    - 133 µl ONPG solution
    - 6 µl magnesium solution
4. Preincubate these reaction mixtures at 37°C by placing them in an appropriate water bath or a heat block
5. Once they've been prewarmed, add
    - 60 µl cell extract
    - it would be wise to also carry out a negative control sample with either extraction instead of cell extract or with cell extract from cells that do not express β-galactosidase
6. Watch for the appearance of a yellow color (*o*-nitrophenol) in each reaction tube; At appropriate timepoints, remove 100 µl aliquots from each reaction mixture and add it to one of the cuvettes containing the sodium carbonate stop solution
7. Once all of the timepoints have been taken, read the optical density at 420 nm; record the absorbance and calculate the slopes of the absorbance over time; the slope is proportional to the β-galactosidase activity; NOTE: A_{420} values above 1.0 should not be trusted
8. To calculate the actual activity, prepare a standard curve of o-nitrophenol and determine the absorbance of this product at each concentration; one unit of enzyme will catalyze the production of 1 µmol of o-nitrophenol per minute at 37°C

Notes on harvesting and lysing cells

Harvesting of Cultures

1. At appropriate time points during the culture, remove 1.6 ml of culture into an Eppendorf tube and place on ice.
2. Working quickly, briefly vortex the 1.6 ml sample and remove 100 µL into a spectrophotometer cuvette containing 900 µL fresh culture media. Spin down the remaining 1.5 ml sample, remove the supernatant and place the pellet immediately at -80°C. (Note: Samples will keep at -80°C indefinitely; do NOT freeze at -20°C.)

3. Measure and note the OD_{600} of the 1:10 dilution of the cell culture. (Note: The OD_{600} of your sample will factor into the later calculation of *lacZ* activity.)

Lysing of Cells

1. Thaw sample pellets on ice.
2. Resuspend pellets in 374 µL B-PER™ Bacterial Protein Extraction Reagent (Pierce product 78248), 50 µL Protease and Phosphatase Inhibitor Cocktail for use with bacterial cell extracts (Sigma product P8465; reconstituted per manufacturer's indications), 1 uL 34 mg/mL chloramphenicol (prepared in methanol), and 6 µL 10mg/ml lysozyme (prepared *fresh* in dH_20; can be omitted when working with *E. coli*).
3. Vortex vigorously for 1 min.
4. Incubate on ice for 5 min.
5. At this point, what you have is a crude lysate slurry that can be assayed for *lacZ* activity using the procedure in the previous section yielding a measure of activity in terms of amount of product formed/minute per OD_{600} unit. (Note: For *Corynebacterium* and *Rhodococcus* this is the best you can hope for using this lysis procedure).
6. For *E. coli* samples, spin down the crude lysate 1 min and place on ice.
7. What you have now is a cleared lysate (the supernatant cleared of cell debris). The protein concentration of the cleared lysate can be determined using the Bradford assay (see the separate protocol for this assay). The cleared lysate can be assayed for *lacZ* activity using the procedure described in the previous section, yielding a measure of activity in terms of nM product formed/minute per mg protein. (Note: Activity per mg protein is a more desirable measure of activity than activity per OD_{600} unit.)

Sodium Phosphate Buffer (50 ml)
49.6 ml distilled sterile water
0.108 g NaH_2PO_4
0.582 g Na_2HPO_4
filter sterilize or autoclave

ONPG solution (10 ml)
10 ml sodium phosphate buffer
0.04 g *o*-nitrophenyl-β-D-glactopyranoside
filter sterilize and store as 1 ml aliquots at -20°C

Magnesium solution (1 ml)
610 µl distilled sterile water
290 µl β-mercaptoethanol
100 µl 1M $MgCl_2$

0.4 M Na_2CO_3 (Stop Solution)
for 500ml:
21.2g Na_2CO_3
dH_2O to 500ml
Prepare ahead and store at room temperature.

10mg/mL lysozyme (1 ml)
(if working with *Rhodococcus* or *Corynebacterium*)
10mg lysozyme
dH2O to 1mL
Prepare *fresh* and keep on ice.

Protease and Phosphatase Inhibitor Cocktail for use with bacterial cell extracts
Sigma product P8465
Reconstituted per manufacturer's indications
Prepare ahead and store 200 uL aliquots at -80°C

34 mg/ml chloramphenical
for 10 mL
340mg chloramphenical
methanol to 10mL
Prepare ahead and store 1 mL aliquots at -20°C

B-PER™ Bacterial Protein Extraction Reagent
Pierce ready-to-use product 78148, 500mL
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