

ASSAY OF β GALACTOSIDASE IN BACTERIAL CULTURES page 33

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http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Lac_Operon_Derepression/Lactase_in_Ecoli.htm

[Review Jacob and Monod's work.] This is a preliminary experiment to learn how to assay levels of β galactosidase in *E. coli* B. From our data, we should be able to answer the question "What are the optimum conditions to assay β galactosidase levels in repressed and derepressed *E. coli*?"

EQUIPMENT FOR CLASS:

clinical centrifuge, balance
 two vortexes set up on side benches

SUPPLIES PER TEAM OF TWO:

5 mL glucose-grown cells, A_{660} at least 0.5
 (CSH $A^1 + 0.1\%$ glu)
 (In shaken 125 mL flasks, **grow 60 mL**)

EQUIPMENT FOR TEAMS OF TWO:

200, 1000 & 5000 uL micropipets & tips
 (One each per table. Are pipets accurate?)
 spectrophotometer, warmed up
 two cuvettes, "B" and "S" in rack
 two 16x150 mm test tubes, with rack
 eleven 13x100 mm test tubes, with rack
 37°C hot block, warmed up
 stopwatch

5 mL lactose-grown cells (CSH $A^1 + 1.0\%$ lac)
 (In shaken 125 mL flasks, **grow 60 mL**)
 2% K_2CO_3 (at least 15 mL/team) in 2mL repipet
 20 mM orthonitrophenyl β D-galactoside
 (1 mL per team, in 13x100 mm)
 0.1 M PO_4 buffer, pH 6.5
 Toluene
 parafilm and scissors
 sterile dH_2O in repipetter

1. Prepare a table in your lab book with the additions you will make to each reaction tube:

| Tube | dH_2O | buffer | growth cond | A_{660} of cells | 20 mM ONPG | Start A_{415} | Finish A_{415} | change ΔA_{415} | Sp.Act: $\frac{A_{415}}{A_{660}}/mL$ |
|------|---------|--------|-------------|--------------------|------------|-----------------|------------------|-------------------------|--------------------------------------|
| R1 | 0.4 | 0.4 | glucose | 3.00 | 0.2 | | | | |
| R2 | 3.3 | 0.4 | lactose | 0.10 | 0.2 | | | | |
| R3 | 2.9 | 0.4 | lactose | 0.50 | 0.2 | | | | |

2. Label eleven 13x100 tubes:

a) 2 culture dilution tubes for the carbon source, glucose or lactose: **Glu and Lac**

b) 3 enzyme Rxn tubes: **R1, R2 & R3**

c) 6 K_2CO_3 tubes: **1S, 1F, 2S, 2F, 3S, 3F** (A Start and Finish for each reaction tube)

3. Spin down 5 mL cells in 16x150 mm tubes in the clinical centrifuge, speed 4 for 10 minutes. Decant and discard supernatant. Resuspend in 10 mL sterile dH_2O . Read A_{660} . Perform dilution to make 4 mL of cell suspension (**Glu and Lac**) with an $A_{660} \approx 0.2$. Read and record A_{660} .
4. **Toluenize the cells:** Add 3 drops of toluene to the diluted cells, cover with parafilm, hold thumb securely over parafilm, shake well and *place on ice*.
5. **Add 2 mL 2% K_2CO_3 to each of the 6 K_2CO_3 tubes.** [Use a repeater pipet.]
6. **Set up enzyme reaction tubes:** Add three ingredients to the enzyme Rxn tubes as in the table in this order: 1) dH_2O (use 5 mL pipet), 2) buffer, 3) toluenized cells. Do *not* add ONPG yet.
7. **Prewarm** the three prepared enzyme Rxn tubes in 37°C hot block for two minutes.
8. **Start the reaction** by adding 0.2 mL ONPG to each tube, vortex, return to 37°C hot block.
9. **Withdraw the starting blanks** (a single 1 mL micropipet may be used for this stage if done in sequence): take 1.00 mL from Rxn tube 1, add to tube 1S (containing 2 mL K_2CO_3), *start stopwatch*. Return tube Rxn1 to 37°C hot block. At 30 second subsequent intervals, take 1 mL from Rxn tube 2 add to tube 2S, then 1 mL from 3 to 3S, each with K_2CO_3 . Flick tubes to mix.
10. **Incubate Rxn tubes 1, 2 and 3 for 15 minute.**
11. **Withdraw the finish samples:** using a fresh tip and in sequence, take 1.00 mL from Rxn tube 1, add to tube 1F. At 30 second subsequent intervals, withdraw 1 mL aliquots from Rxn tube 2, add to 2F, repeat with Rxn tube 3 to tube 3F. Flick tubes to mix.
12. **Read and record the A_{415} of the six tubes** (1S, 1F, 2S, 2F, 3S, 3F). (REMEMBER: A_{415} !)
13. Determine the change in A_{415} (ΔA_{415}) for each of the three enzyme reaction tubes.
14. **Calculate specific activities** for each sample:

(1/mL aliquot of cells tested) x (ΔA_{415} from assay)/(A_{660} of the diluted culture)

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REAGENTS FOR LACTASE ENZYME ASSAY:

0.1 M PO_4 pH 6.5 buffer:

0.5 g KH_2PO_4 + 0.9 g Na_2HPO_4 , dissolve in 100 mL H_2O

20 mM o-nitrophenyl- β -D galactoside (ONPG):

181 mg ONPG, dissolve 30 mL dH_2O , warm slightly & swirl to dissolve.

2% K_2CO_3 :

dissolve 5 g K_2CO_3 in 250 mL dH_2O , stir to dissolve.
 (Or dilute 4% 1:1.)

10x Cold Spring Harbor A Medium

$(NH_4)_2SO_4$ 10 g
 K_2HPO_4 105 g
 KH_2PO_4 45 g
 $MgSO_4 \cdot H_2O$ 1g
 dissolve in dH_2O , q.s. to 1 L, autoclave

dilute 1:10 into sterile dH_2O
 add sterile carbon source after dilution