ASSAY OF β GALACTOSIDASE IN INDUCED BACTERIAL CELLS

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

Your team should now have five samples of toluenized cells: 0, 20, 40, 60 and 80 minute lactose-grown from the previous stage of the experiment. Last week's prelimiary experiment demonstrated the approximate repressed and derepressed levels of β galactosidase and optimum assay conditions for these toluenized cells. You will now assay the amount of β galactosidase in this set of cells using a similar protocol, and then calculate the specific activity to reveal derepression.

EQUIPMENT per desk of two:

- 15x 13 x 100 mm test tubes in TT rack
- 1x 37°C hot block, thermometer
- 1x 5 mL displacement pipetes & tips
- 2x 1 mL micropipets & tips
- 1x stopwatch
- 1x spectrophotometer
- 2x cuvettes, in TT rack

SUPPLIES (per 16 students):

- your 5 samples of toluenized cells on ice: T-0, T-20, T-40, T-60, T-80 (Minutes grown in lactose)
- 350 mL 2% K_2CO_3 in 2.0 mL repipet
- 20 mL 20 mM orthonitrophenyl galactoside
- 50 mL 0.1 M PO_4 buffer, pH 6.0, 0.4 repeater
- 25 mL dH_2O (in 125 mL flask)

1. **Label tubes, per team of two:**
   - five Rxn tubes: R-0 , R-20, R-40, R-60, R-80
   - ten K_2CO_3 tubes, start and finish: S0, S20, S40, S60, S80, F0, F20, F40, F60, F80
2. **Add 2 mL 2% K_2CO_3 to each of the S and F tubes with repeater pipet.**
3. **Set up the assay table.** Set up the following table in your notebook (2x or 4x spaced)

tube	sample	A_{660}	dH_2O	buffer	toluenized cells		A_{415}	F - S	$\Delta A_{415}/A_{660}$/aliquot
R-0	T-0		--	0.4	3.2	S			
		_____				F			
R-20	T-20		- -	0.4	3.2	S			
		_____				F			
R-40	T-40		2.2	0.4	1.0	S			
		_____				F			
R-60	T-60		2.7	0.4	0.5	S			
		_____				F			
R-80	T-80		2.7	0.4	0.5	S			
		_____				F			

4. **Set up reaction tubes:** add water, then buffer (with repeater pipet), then toluenized cells. Mix, place in 37°C hot block to pre-warm. Place a thermometer in tube R-0, wait until temp = 35°C. Because the large sample of cold cells in tubes R-0 and R-20, we will start the reaction in R-80 first, then R-60, etc. while tubes R-0 and R-20 continue to warm up.
5. **Add 0.4 mL ONPG down the side of all five R tubes, vortex.**
   Then begin taking Start samples: *at 30 second intervals:*
   - a: **Withdraw 1.0 mL start sample** from R-80 assay tube, add to the S-80 tube (contains K_2CO_3), mix, and **start stopwatch**. Place S-80 in rack to be read later. Wait 30 seconds.
   - c: **Withdraw 1.0 mL R-60 tube**, add to S-60 exactly 30 seconds after the previous sample. Repeat for remaining reaction tubes, R-40, R-20 and R-0.
   - d: **Incubate** the five assay tubes for exactly15 minutes each at 37°C.
   - e: **When the stopwatch reads 15 minutes, withdraw 1.0 mL from R-80**, add to F-80 (with K_2CO_3), mix. At 30 second intervals, repeat aliquoting to F-60, F-40, F-20 and F-0
4. **Read the A_{415} of all tubes, reading S-0 to S-80 tubes first, then the F-0 to F-80 last**. Subtract the start from the finish = ΔA_{415} (which is proportional to the amount of enzyme present).
5. **Calculate specific activities** for each sample:
   (1/mL of cells tested) x (ΔA_{415} from assay)/(A_{660} of the culture at sampling time)
6. **Graph the specific activities of the two cultures versus time.** Discuss differences in specific activity seen as the culture grows for progressively longer times in lactose.