

PROTOCOL FOR LINEWEAVER-BURK PLOT: LACTASE KINETICS page 31

David B. Fankhauser, PhD

8Nov94, rvsd 17Sept95, 29Oct96, 20Sept99, 30Nov99, 18Sept00, 28Nov00, 22Sept03, 25Nov03, 20Sept04, 24Nov09, 23Nov10
http://biology.clc.uc.edu/fankhauser/Labs/Cell_Biology/Lineweaver_Burk_Plot/Lactase_lineweaverBurk.htm

Equipment per team of two:

19x *clean* 13 x 100 mm tubes in rack:
 (label them: B, 1-6, 11-16, 21-26)
pipettors: 1x 200 λ (ONPG)
 (and tips) 1x 1000 λ (H₂O, ONPG, K₂CO₃)

vortex
 37°C hot block, 13 mm holes, preheated
 250 mL beaker for used tips

Supplies: (1 set per team of two)

125 mL flask with about 30 mL dH₂O
 16 x 150 test tube with 10 mL 20 mM ONPG
 three Rxn Mixes (see below)
 50 mL beaker with 25 mL 4% K₂CO₃

Additional equipment:

for class: 3x: repeat pipetter (Rxn Mix)
 stopwatch
 warm spectrophotometer, 2 cuvettes & wipettes

		Final concentration: 25 mM 5 mM	
		Set up to deliver at 3 stations: glucose galactose	
Total RxnMx Per 7 teams: mL		Supplimented RxnMx	
dH ₂ O	42	control	glucose
0.1 M PO ₄ , pH 5.5	210	Rxn Mix	galactose
100 U/mL lactase	0.05	mL 1.0 M sugar	
Total RxnMx:	252	dH ₂ O	
		Final vol:	
		86.1	86.1
		86.1	86.1

Cut here.....

Experiment Table: Enter this table into your notebook, then number your tubes, set up in a rack, add the ingredients as specified in the steps below. Fill in A₄₅₀ and calculat'ns as you work thru expt.

	tube	uL	mL	uL	mL	A 450	1/[S]	1/A450
		dH ₂ O	Rxn Mx	ONPG	K ₂ CO ₃	x1000:		
no add	B	800	1.2	0	1			
	1	670	1.2	130	1			
	2	610	1.2	190	1			
	3	530	1.2	270	1			
	4	410	1.2	390	1			
	5	240	1.2	560	1			
	6	0	1.2	800	1			
glucose	11	670	1.2	130	1			
	12	610	1.2	190	1			
	13	530	1.2	270	1			
	14	410	1.2	390	1			
	15	240	1.2	560	1			
	16	0	1.2	800	1			
galact	21	670	1.2	130	1			
	22	610	1.2	190	1			
	23	530	1.2	270	1			
	24	410	1.2	390	1			
	25	240	1.2	560	1			
	26	0	1.2	800	1			

PROTOCOL:

- 1) **Add dH₂O to labeled tubes:** (*i.e.*, the volume required to *q.s.* to a final volume of 2.0 mL).
- 2) **Deliver 1.20 mL of appropriate Rxn Mix to each tube (repeat pipeter). Use a separate pipetter for each of the three RxnMixes. Prewarm to 37°C, two minutes.**
- 3) **Add ONPG:** At 15 second intervals, carefully add 130 uL ONPG and vortex to tubes 1 then 11, then 21. Then change volume to 190 uL and add to 2, 12, 22, etc until all tubes are started. (Deliver to the side of the reaction tube just above but not touching the surface, avoid the possibility of picking up lactase and taking it back to the ONPG). Vortex the tube to pick up all of the ONPG which was deposited on the side of the tube. Incubate all tubes at 37°C.
- 4) **After exactly 15 minutes, add 1 mL of 4% K₂CO₃ and mix** at the same regular intervals to reaction tubes to halt the reaction. (*I.e.*, each tube should be incubated *exactly* 15 min.)
- 5) **Read A₄₅₀ for each tube** against the B tube (contains no ONPG).
- 6) **Graph on a linear scale** to produce a *Substrate Saturation Curve*.
- 7) **Fill in the table** of the values for the inverses of substrate concentrations and of enzyme velocities.
- 8) **Graph the inverses to produce Lineweaver-Burk plot.** What is the V_{max}? What is the K_m?

INHIBITION EXPERIMENT: Analyze the class of inhibition (if any) of glucose or galactose: add final conc of 25mM glucose or 5mM galactose. Test for inhibition of ONPG digestion.

Reaction mix:	control	glucose	galactose
dH ₂ O	1.9 mL	1.4 mL	1.8
0.1 M PO ₄ buffer, pH 7.0	10.0 mL	10 mL	10.0 mL
vol 1 M sugar to be tested	n/a	0.5 mL, 1.0 M*	0.1 mL, 1.0 M*
lactase, 30 FCC units/mL	100 µL	100 µL	100 µL

* (1.0 M hexose = 7.92 g/40 mL. Keeps well frozen, but galactose precipitates out. Heat to dissolve.)

What kind of inhibition, if any, is demonstrated?