

LACTASE pH OPTIMUM

page 28

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Enzymatic activity is strongly dependant on protein conformation. Since pH can determine whether an amino acid's side chain is charged or not, and ionic interactions strongly affect tertiary protein structure, pH has a pronounced effect on a protein's conformation and therefore on its catalytic activity. Typically, the maximum rate of action of an enzyme is found only when it is folded in a precise fashion. The pH which produces this precise folding is termed its pH optimum. An enzyme's pH optimum may be determined by performing multiple assays, each identical except for the pH at which it is run. Graphic display of the resulting data (rate versus pH) demonstrates the enzyme's pH optimum. Here we will determine the pH optimum of the enzyme lactase.

In the preparatory stage of this experiment, an array of buffers have been formulated which cover the pH range to be tested. Typically this can be done by preparing two stock buffers (one acidic, the other basic) which, when mixed together in varying proportions, yield varying pHs. The two stock buffers which we will use are: 1) boric acid/citric acid and 2) Na_3PO_4 . Varying their ratios produces pHs ranging from 2 to 9. For the preparation of these buffers and their proportions for desired pH, see *Chemical Technicians' Ready Reference Handbook*, p. 656-657.

As in many enzyme assays, adjustments in concentrations and volumes may be needed for optimum results. Keep careful track of how you set up your experiment.

MATERIALS AND EQUIPMENT: (PER TEAM OF TWO STUDENTS)

0.1 units/mL enzyme dilution, 100 mL total. Prep: displ. pipetters, 0.2 & 1.0 mL
0.1 mL lactase susp'n (100 units/mL), q.s. w/ dH_2O to 100 mL 37°C hot block, 13 mm holes
20 mM *o*-nitrophenyl- β -D galactoside (3.0 mL ONPG each team) thermometer
series of buffers of noted pH made from vortex
boric acid/citric acid + Na_3PO_4 varying ratios (note *actual* pH) stopwatch
test tubes: 12 clean 13x100 mm in rack, labeled 4% K_2CO_3
repeater pipetter with 10 mL chamber for adding 0.8 mL enz dil. spectrophotometer
two cuvettes in rack at spec.

COPY THIS TABLE INTO YOUR NOTEBOOK. (Check for correct pH figures.)

tube:	mL 0.1 M buffer	pH of buffer	mL dil enz	20 mM ONPG	final volume	A_{450} :
B		1.8 dH_2O	--		2.0	
1	1.0	2.0	0.80	0.2	2.0	
2	1.0	2.7	0.80	0.2	2.0	
3	1.0	3.5	0.80	0.2	2.0	
4	1.0	4.2	0.80	0.2	2.0	
5	1.0	5.0	0.80	0.2	2.0	
6	1.0	5.7	0.80	0.2	2.0	
7	1.0	6.5	0.80	0.2	2.0	
8	1.0	7.0	0.80	0.2	2.0	
9	1.0	7.5	0.80	0.2	2.0	
10	1.0	8.0	0.80	0.2	2.0	
11	1.0	8.5	0.80	0.2	2.0	

1. **Add enzyme dilution** (0.8 mL) *down the side* of each tube, using repeat pipetter.
2. **Add specified buffer** (1 mL each) to its appropriate tube. You may use the same pipet tip *if* you progress in sequence up through the buffers for all ten tubes, blowing out and tipping off any clinging droplets. Cross contamination effects should be minimal. Vortex holding tube near the top to wash down the sides.
3. **Pre-warm these tubes** in a 37°C hot block for two minutes.
4. **Start the reaction** at 15 second intervals: **add 0.2 mL ONPG**, vortex, start a stopwatch with 1st tube, replace each tube in succession in 37°C hot block.
5. **Stop the reaction** after exactly 15 minutes by adding 1.0 mL 4% K_2CO_3 down the side of the first tube, mix and remove from hot block. At 15 second intervals, repeat the addition of 4% K_2CO_3 for each of the successive tubes, mix and transfer to the test tube rack.
6. **Read the absorbency at 450 nm**, record in your notebook, graph and discuss results.