

ENZYME ASSAY: LACTASE

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27Sept93, rvsd 25Oct94, 17Sept95, 22Oct96, 20Sept99, 18Sept00, 17Oct02, 21Oct03, 19Sept04, 15Oct07, 21Oct08, 20Oct09

[See Hartman, Suskind & Wright, *Principles of Genetics Lab Manual*, (1965). pp. 52-58.]
http://biology.clc.uc.edu/fankhauser/Labs/Cell_Biology/Lactase/Lactase_Assay.htm

Lactose, (milk sugar) is a disaccharide formed from galactose and glucose. Before these sugars can be absorbed by the body, its β -galactosidic bond must be hydrolyzed to the monosaccharides. In humans, the enzyme lactase performs this task. Many individuals lose the ability to digest lactose as they enter their teens, and may for this reason suffer GI upset when they consume milk products. Lactase tablets may reduce this problem. We will be assaying the lactase content in these tablets, and use this enzyme as a typical enzyme in our subsequent studies of enzymes.

The substrate used in the assay of this enzyme is *o*-nitrophenyl- β -D galactoside (ONPG), which, upon hydrolysis of the β -galactosidic bond, yields galactose and *o*-nitrophenol, a yellow compound (absorption max = 450 nm) (*CRC Handbook*: #p679, *Merck Index*, #6541). Enzyme activity is proportional to the increase in A_{450} during a timed incubation due to ONPG produced.

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 assay)

20 mM <i>o</i> -nitrophenyl- β -D galactoside (1.2 mL ONPG)	37°C hot block, 13 mm holes
0.1 M PO ₄ buffer, pH 5.5 (6 mL)	vortex
0.01 M PO ₄ buffer, pH 5.5 (120 mL)	stopwatch
200 λ and 1000 λ micropipets and tips	4% K ₂ CO ₃
test tubes: one 16x150, five 13x100 in rack	spectrophotometer
25 mL and 100 mL graduated cylinders	cuvettes in rack at spectrophotometer

1. **Record** the brand of lactase, labeled number of units of lactase/tablet and the expiration date.

Weigh one lactase tablet, note whether 9,000 FCC or 3,000 FCC units/tablet.

Grind in a mortar and pestle until finely ground.

Suspend/dissolve to 100 units/mL: Grind a tablet in about 5 mL of chilled 0.01 M PO₄ buffer, pH 7. **For 9,000 unit tabs, q.s.** to 90 mL with same buffer, including rinses of mortar and pestle.

For 3,000 unit tab, q.s. to 30 mL (Solution will be cloudy because of undissolved binder.)

Dilute 1:200 (to 0.5 units/mL): Add 100 μ L of enzyme suspension into 19.9 mL 0.01 M PO₄ in a 25 mL grad cylinder. Most enzymes are kept on ice until use, but not necessary for lactase.

2. Copy the following table into your notebook.

3. Set up a series of numbered 13x100 mm test tubes as in table below.

4. Prepare the reaction mix (Rxn Mix) per assay set:
5.6 mL 0.1 M PO₄, pH 5.5
1.4 mL 20 mM ONPG

5. **Add water to each tube first, then Rxn Mix. (Not the enzyme yet.):**

tube:	mL dH ₂ O	mL Rxn Mix	μ L diluted enzyme	mL total volume	A_{450}
1 (blank)	1.00	1.0	0	2.0	
2	0.975	1.0	25	2.0	
3	0.95	1.0	50	2.0	
4	0.90	1.0	100	2.0	
5	0.80	1.0	200	2.0	

6. Pre-warm these tubes in a 37°C hot block for two minutes.

7. Add listed μ L of enzyme to tube no. 2, vortex, **start a stopwatch**. Add enzyme to successive tubes at 30 second intervals, vortex each in turn and **replace in 37°C hot block**.

8. At about 14.5 minutes, add 1.0 mL 4% K₂CO₃ down the side of tube no. 1 (the blank), mix and remove from hot block. At exactly 15 minutes, add 1.0 mL 4% K₂CO₃ to tube 2, mix and place in test tube rack. At 30 second intervals, repeat this operation for each of the successive tubes.

9. Using tube 1 as blank, read the absorbency at 450 nm of all tubes, record in the table in your notebook, graph and discuss results.

10. Calculate the number of units of lactase (1.000 OD unit/15 min) in the original tablet. (See following protocol). Compare with other brands of lactase.

19 September 1993, rvsd 25 October 1994, 18 Sept 95, 20 Sept. '96

0.1 M PO₄ pH 5.5 BUFFER:

For 200 mL, weigh out: 1.2 g KH₂PO₄
1.2 g Na₂HPO₄

dissolve in 200 mL H₂O, check pH, adjust to 7.0 if nec. with either H₃PO₄ or NaOH. Store at 4°C.

0.01 M PO₄ pH 5.5 BUFFER: (for suspension and dilution of enzyme)

Q.s. 50 mL of pH 5.5 0.1 M PO₄ buffer to 500 mL with dH₂O.

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

Weigh out: 602 mg ONPG

dissolve in about 80 mL 0.01 M PO₄ buffer, pH 7.0 with swirling and slight warming. *q.s.* with buffer to 100.0 mL.] E₄₁₀ = millimolar extinction coefficient of o-nitrophenol (3.5 mM-1cm-1)

REAGENT TO HALT REACTION:

4% K₂CO₃: dissolve 8 g K₂CO₃ in 200 mL dH₂O, stir to dissolve.

MATERIALS AND EQUIPMENT for team of four assaying given brand of lactase:

(two sub teams each perform an assay) 10/25/94, rvsd 18 Sept '95, 20 Sept. '96, 15Oct07

EQUIPMENT:

mortar and pestle
100 mL graduated cylinder
ice bath
5.0 mL pipet (for dH₂O)
pipet bulb or helper
2 x 200 lambda micropipettes
(for ONPG and enzyme)
2 x 1000 lambda micropipettes
(for buffer and 4% K₂CO₃)
2 16 x 150 mm test tubes
10 13 x 100 mm tubes
two test tube racks for 13x100 tubes
37°C hot block for 13 x 100 mL
2 stopwatches
spectrophotometer, warmed up
at spectrophotometer:
cuvettes in rack
wipettes

SUPPLIES:

lactase tablets
100 mL 0.01 M PO₄pH 5.5 buffer
(to suspend and dilute enzyme)
30 mL dH₂O in 125 mL flask
(to make up assay set)
3 mL 20 mM o-nitrophenyl-β-D galactoside
(ONPG)
15 mL 0.1 M PO₄ buffer, pH 5.5
(for assay tubes)
15 mL 4% K₂CO₃

CALCULATION OF LACTASE ACTIVITY/TABLET:

If 1 unit of lactase produces an OD of 1.000/15 min., and the assay was run for 15 mins:

$$\text{units/tablet} = A_{450} \times \text{mL/tablet suspension} \times \text{dilution factor} \times 1/(\text{aliquot in mL})$$