

# LACTASE: COMPARISON OF CONTENT IN BRANDS page 27

David B. Fankhauser, PhD

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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: For a class of 16 ( 8 teams of two students each)

variety of commercial lactase tablets	Eppendorf repeater pipet, 50 mL tip
20 mM <i>o</i> -nitrophenyl- $\beta$ -D galactoside (40 mL)	8 37°C hot blocks, 13 mm holes at desks
0.1 M PO <sub>4</sub> buffer, pH 5.5 (200 mL)	8 vortexes at desks
0.01 M PO <sub>4</sub> buffer, pH 5.5 (1000 mL)	8 stopwatches at desks
8 each: displacement pipets: 200 $\lambda$ , 1000 $\lambda$ and tips	200 mL 4% K <sub>2</sub> CO <sub>3</sub>
8 sets test tubes: 20 13x100mm in rack	8 spectrophotometers (on side benches)
8 sets beakers: 250 mL and a 80 mL	2 cuvettes in a rack at each spectrophotometer

- Record:** your brand of lactase, expiration date and labeled number of units of lactase/tablet.

**Weigh:** one lactase tablet, 9,000 units per tab. \*

**Grind:** in a mortar and pestle until finely ground.

**Suspend/dissolve 100 units/mL:** Susp in about 5 mL of 0.01 M PO<sub>4</sub>, pH 5.5, *q.s.* to 90 mL  
(Solution will be cloudy because of undissolved starch binder.)

**Dilute 1:200:** Add 0.1 mL of suspension to 19.9 mL of 0.01 M PO<sub>4</sub>, pH 5.5

**Distribute** 1 mL of the 1:200 enzyme into each of 4 labeled tubes, distribute one to every row of tables.  
(Each row gets an aliquot of every enzyme prep, the two tables share enzyme set.)

- Copy the following table into your notebook. Adjust for class size. Fill in name of brand.

tube: team	Commercial Brand	volume Rxn mix	volume dil enz	A <sub>450</sub> :	average of duplicates
B	0.1 mL dH <sub>2</sub> O	2.0	--	0.000	_____
1 1	Brand A suspension	2.0	0.1	_____	_____
2 1	Brand A suspension	2.0	0.1	_____	_____
3 2	Brand B suspension	2.0	0.1	_____	_____
4 2	Brand B suspension	2.0	0.1	_____	_____
5 3	Brand C suspension	2.0	0.1	_____	_____
6 3	Brand C suspension	2.0	0.1	_____	_____
7 4	Brand D suspension	2.0	0.1	_____	_____
8 4	Brand D suspension	2.0	0.1	_____	_____
9 5	Brand E suspension	2.0	0.1	_____	_____
10 5	Brand E suspension	2.0	0.1	_____	_____
11 6	Brand F suspension	2.0	0.1	_____	_____
12 6	Brand F suspension	2.0	0.1	_____	_____

- Set up a series of numbered 13x100 mm test tubes as listed, one per student in the class + blank.

- Prepare reaction mix (Rxn mix)** mL/tube      mL for a class of 16:

dH <sub>2</sub> O	0.7	119
0.1 M PO <sub>4</sub> pH 5.5 buffer	1.0	170
20 mM ONPG	0.2	34

Swirl to mix well

- Aliquot 2.0 mL of Rxn mix to each tube with a repeater pipet. Do **not** add the enzyme yet!
- Pre-warm these tubes in a 37°C hot block for two minutes.
- At T<sub>0</sub>, add 0.1 mL of the first diluted enzyme, vortex, start a stopwatch with 1st tube. At 30 (15?) second intervals, add the appropriate enzyme to each successive tube.
- After exactly 15 minutes, add 0.9 mL 4% K<sub>2</sub>CO<sub>3</sub> down the side of the first tube (B), vortex and remove from hot block. At 30 (15 sec?) second intervals, repeat addition of 4% K<sub>2</sub>CO<sub>3</sub> for each of the successive tubes, vortex and place in a test tube rack.
- Read the absorbency at 450 nm, record in your notebook, average the duplicate tubes.
- Calculate the number of units of lactase (1.000 OD unit/15 min) in the original tablets. (See previous protocol). Compare with other brands of lactase, and make a bar graph to compare.

\* 3,000 unit tablet: susp in about 5 mL of 0.01 M PO<sub>4</sub>, pH 5.5, *q.s.* to 30 mL