

LACTASE: COMPARISON OF CONTENT IN BRANDS

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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: (per team of two students, class of 12)

variety of commercial lactase tablets	Eppendorf repeater pipet, 50 mL tip
20 mM <i>o</i> -nitrophenyl- β -D galactoside (20 mL)	37°C hot block, 13 mm holes
0.1 M PO ₄ buffer, pH 7.0 (100 mL)	vortex
0.01 M PO ₄ buffer, pH 7.0 (120 mL)	stopwatch
displacement pipets: 5 mL, and 200 λ and 1000 λ	30 mL 4% K ₂ CO ₃
test tubes: six 16x150, 13 13x100mm in rack	spectrophotometer
beakers: 250 mL and a 80 mL	cuvettes in rack at spectrophotometer

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

Weigh one lactase tablet.

Grind in a mortar and pestle until finely ground.

Suspend/dissolve: 9,000 unit tablet: susp in about 5 mL of 0.01 M PO₄, pH 7, *q.s.* to 90 mL
(To 100 units/mL) 3,000 unit tablet: susp in about 5 mL of 0.01 M PO₄, pH 7, *q.s.* to 30 mL
(Solution will be cloudy because of undissolved binder.)

Dilute 1:50: Add 0.1 mL of suspension to 4.9 mL of 0.01 M PO₄, pH 7

2. Copy the following table (for class of 12) into your notebook. Fill in name of brand.

tube:	Brand and number	Commercial volume	volume of reaction mix	dil enzyme	A ₄₅₀ :
B		0.1 mL dH ₂ O	2.0	--	
1	Brand A	suspension 1	2.0	0.1	
2	Brand A	suspension 1	2.0	0.1	
3	Brand A	suspension 2	2.0	0.1	
4	Brand A	suspension 2	2.0	0.1	
5	Brand B	suspension 1	2.0	0.1	
6	Brand B	suspension 1	2.0	0.1	
7	Brand B	suspension 2	2.0	0.1	
8	Brand B	suspension 2	2.0	0.1	
9	Brand C	suspension 1	2.0	0.1	
10	Brand C	suspension 1	2.0	0.1	
11	Brand C	suspension 2	2.0	0.1	
12	Brand C	suspension 2	2.0	0.1	

3. Set up a series of numbered 13x100 mm test tubes as listed.
4. Prepare **reaction mix (Rxn mix)** in 25 mL beaker to be distributed to the tubes: [mL/tube]
dH₂O 9.0 mL (10 mL graduated cylinder) fr 6 suspnsn: [54] 0.7
0.1 M PO₄ pH 7.0 buffer 15.0 mL (25 mL graduated cylinder) [90] 1.0
20 mM ONPG 3.0 mL (5 mL displacement pipetter) [18] 0.2
Swirl to mix
5. Aliquot 1.9 mL of Rxn mix to each tube with a repeater pipet. Do **not** add the enzyme yet!
6. Pre-warm these tubes in a 37°C hot block for two minutes.
7. At 30 second intervals, add listed volume of enzyme, vortex, start a stopwatch with 1st tube.
8. After exactly 15 minutes, add 0.9 mL 4% K₂CO₃ down the side of the first tube (B), vortex and remove from hot block. At 30 second intervals, repeat addition of 4% K₂CO₃ for each of the successive tubes, mix and set aside.
9. Read the absorbency at 450 nm, record in your notebook, average the duplicate tubes.
10. 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.