REAGENTS, MATERIALS AND CALCULATIONS FOR LACTASE ASSAY


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. \[ E_{410} = \text{millimolar extinction coefficient of o-nitrophenol (3.5 mM-lcm}^{-1}) \]

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, revsd 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:

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