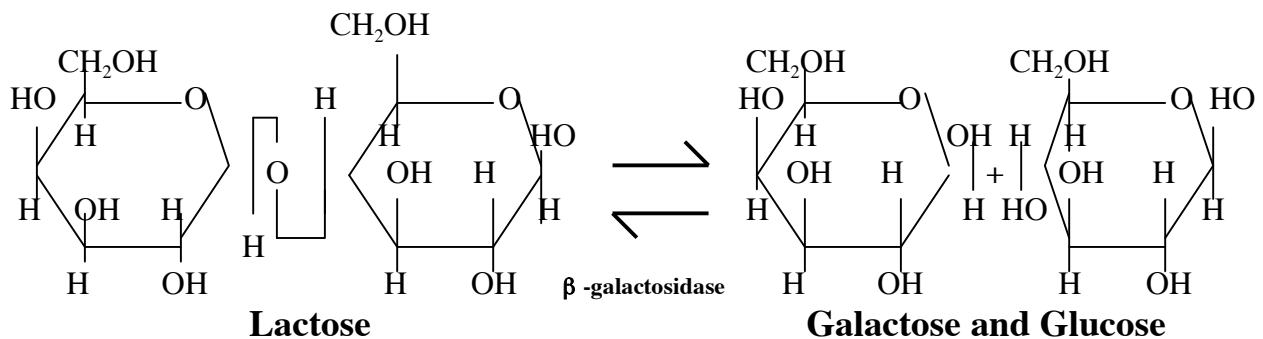


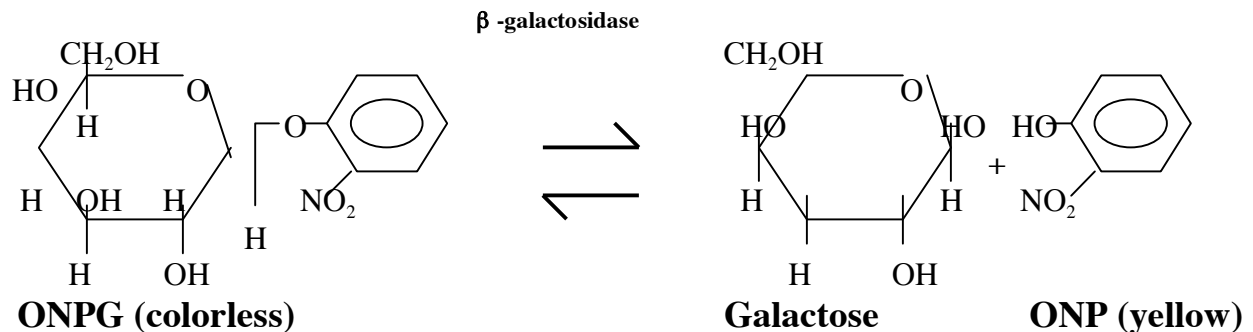
Enzyme assay using β -galactosidase

Lactase, also known as β -galactosidase, catalyzes the hydrolysis of β -galactosides. A naturally occurring substrate of lactase is the disaccharide lactose (milk sugar) that is composed of the monomers β -galactose and glucose.

β -galactosidase is used by bacteria to split lactose into these monomers which can then be utilized by the cell as energy sources. In humans a deficiency in lactase activity causes lactose intolerance where lactose is not broken down and the accumulation of lactose in the intestine provides bacteria with a food source. This results in gas, bloating, and stomach discomfort. Commercially available products such as Lactaid® and Lacteeze® contain lactase and are used by lactose intolerant individuals to help them digest lactose.



One way to determine the activity of lactase is by using the synthetic substrate ONPG (ortho-nitrophenyl- β -D-galactoside). ONPG is useful as an assay material because, although it is colorless, the product of its hydrolysis is yellow. Thus, we can measure the rate at which the product accumulates by measuring the appearance of yellow color. Lactase hydrolyzes ONPG to release galactose and ONP (ortho-nitrophenol). Under alkaline conditions ONP is yellow, so the more ONP present the intenser the yellow color.



Preparation.

Enzyme:

Grind one Lactaid[®] tablet with a mortar and pestle and add to 100 ml of dH₂O. Filter to get out particulate matter. Keep cold until ready to use.

ONPG (substrate):

Make up 1.0 mM ONPG in dH₂O just before lab (0.03g/100 ml). May also try using 5.0 mM ONPG.

ONPG (o-nitrophenyl- β -D-galactosidase), may be purchased from Sigma Chemical Co. (Sigma #N-1127), 1-800-325-5052)

Basic Reaction.

Set the spectrophotometer to 400nm. Blank with 4.5 ml dH₂O + 0.5 ml Lactaid[®] solution.

For the standard reaction add 3.0 ml of 1.0 mM ONPG to a spec tube with 1.5 ml dH₂O. Add 0.5 ml of your enzyme (Lactaid[®]) solution. Mix quickly (this is time zero). Place this tube into the spectrophotometer and take absorbance readings every 15- 30 seconds for three minutes.

Variations:

Temperature – use same volumes as above, but equilibrate all components to the temperature you want to test for at least 10 minutes. Add together. Be sure to put the cuvette back into temperature being tested between readings. You might want to take readings every minute for this reaction.

Substrate concentration – Vary the volumes of 1.0 mM ONPG and dH₂O, but make sure they add up to 4.5 ml (2.0 ml ONPG + 2.5 ml dH₂O, 1.0 ml ONPG + 3.5 ml dH₂O, 4.0 ml

ONPG + 0.5 ml dH₂O, etc.). Then add 0.5ml of enzyme (Lactaid® soln). Take readings every 15-30 seconds.

Sample data showing the rate of the reaction at different substrate (ONPG) concentrations.

