

β -Galactosidase

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Summary

β -galactosidase catalyzes the hydrolysis of β -D-galactosides, such as the conversion of lactose to galactose and glucose. Galactosides modified with compounds that are chromogenic can be used to quantify the activity of β -galactosidase. The most common modified substrate used is *o*-nitrophenyl- β -D-galactoside, which itself is colorless, but which generates the light yellow product *o*-nitrophenyl in the presence of the enzyme. The reaction is terminated by a concentrated Na_2CO_3 which raises the pH to about 11. The time that the reaction took place must be accurately recorded.

Most other enzyme assays require the preparation of the cell extract. For this assay, it is sufficient to shake the bacteria with chloroform. Chloroform disrupts the membrane, allowing small molecules to diffuse from the cell into the medium, while large enzymes (such as β -galactosidase) stay inside the cell.

Solutions Required

1. Z buffer (in a 1.0 L solution):

16.1 g $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$

5.5 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$

0.75 g KCl

0.246 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

can be prepared in stock solution and stored in refrigerator.

2. Modified Z buffer

10 mL Z buffer

27 μL 1% SDS

27 μL β -mercaptoethanol

must be prepared fresh

3. 100 mM potassium phosphate buffer (pH 7.0)

This buffer is made by mixing 12.3 mL 100 mM K_2HPO_4 with 7.7 mL 100 mM KH_2PO_4 .

4. ONPG

Dissolve 4 mg *o*-nitrophenyl- β -D-galactoside in 1.0 mL of 100 mM potassium phosphate buffer (pH = 7.0).

5. 1.0 M Na_2CO_3

6. chloroform

Cell Preparation

1. Thaw frozen cell sample and place on ice. The cells will have to be diluted, but the dilution necessary depends on the particular experiment. When *E. coli* with pACYC-*lacZ* is used, we have found that an OD of 10 must be diluted by a factor of about 500.

Spectrophotometer

Turn on the visible bulb on the spectrophotometer (Beckman DU50). Use a wavelength of 420 nm.

Procedure

1. In a culture tube, mix 100 μL of cell sample with 900 μL modified Z buffer.
2. Add 50 μL chloroform to each sample and vortex.
3. Place samples in water bath at 30°C for 5 minutes.
4. Add 200 μL ONPG to each sample in the water bath. Remove from bath to vortex, commence timer, and return to bath. If multiple samples are being analyzed, it is convenient to start the timer for the first sample, and then permit a 30 second or one minute delay between the initiation of each reaction.
5. Watch for development of yellow color in samples.
6. When the samples are light yellow, add 500 μL Na_2CO_3 to a sample and vortex to terminate reaction. Record reaction time. The reaction time should be between 8 minutes and 30 minutes.
7. Let the culture tube stand for about 1 minute to allow the cell debris to settle.
8. Transfer 1.0 mL of sample into cuvette, avoiding any chloroform or cell debris.
9. Record absorbance at 420 nm. The absorbance should be within the range of 0.3 to 0.9.

Calculation of Activity

One unit (U) of β -galactosidase activity is defined as the amount of enzyme required to produce 1.0 μmole of *o*-nitrophenol in one minute.

$$1. \quad \text{Activity} = \frac{1000 \times TV \times A \times D}{\epsilon \times V \times T}$$

- Activity: Volumetric Activity (U/L)
TV: Total volume (1750 μL)
V: Volume of cell-containing media used (100 μL)
 ϵ : Molar extinction coefficient for *o*-nitrophenol (7.5 mL/ μmol for a path length of 1.0 cm)
T: Reaction time (minutes).
D: Dilution of original sample (For example, if 100 μL of sample is 9.90 mL of deionized water, then D=100)

2. Specific Activity = $\frac{\text{Activity}}{\text{Protein Concentration}}$

Activity: Volumetric Activity, as calculated in #2 above (U/L)
Protein Concentration: Protein concentration, as calculated in protocol **Total Protein Concentration** (mg/L)
Specific Activity: (U/mg protein)

Reference

A. B. Pardee, F. Jacob, J. Monod (1959) "The Genetic Control and Cytoplasmic Expression of Inducibility in the Synthesis of β -galactosidase by *E. coli*," Journal of Molecular Biology 1, 165-178.