

Determination *in vitro* of B-gal activity using Xgal

Introduction

X-Gal is a widely used compound to test the presence of β -galactosidase. It gives a dark blue precipitate at the site of enzymatic activity. It is useful for detection of *lacZ* activity in cells.

Aim: Test how different concentrations of b-gal efficiently cut X gal and after how many minutes a color change can be observed.

Materials

- B-gal enzyme
- X-gal (0.2 mg/ml) dissolved in Dimethylformamide (DMF)
- sterile H₂O
- spectrophotometer

Procedure

1. A volume of 12 ml of 0,2 mg/ml X gal in sterile H₂O was made as a stock solution.
2. A total of 7x 1,5ml cuvettes were filled with the stock solution; six to be analyzed with different conc. B-gal and one to act as a blank.
3. The reading was done at OD 610nm.
4. To six of the cuvettes were added 0.5, 1.0, 1.5, 2.0, 2.5 and 3 μ L B-gal respectively.
5. Each of the solutions were analyzed by a spectrophotometer every 5 min for the first 10 and then at intervals of 2.5 min. The results were composed in the table.
6. Visual checks were also performed at each of the minute marks to see at what point a color change was visible to the human eye.

Note: This protocol can be used for ONPG substrate as well.