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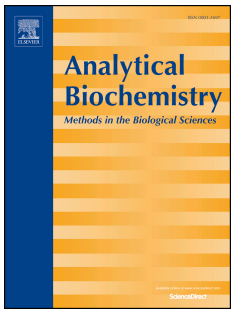

Single-step method for β-galactosidase assays in *Escherichia coli* using a 96-well microplate reader

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Single-step method for β-galactosidase assays in *Escherichia coli* using a 96-well microplate reader

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Abstract

Historically, the *lacZ* gene is one of the most universally used reporters of gene expression in molecular biology. Its activity can be quantified using an artificial substrate, o-nitrophenyl-beta-D-galactopyranoside (ONPG). However, the traditional method for measuring LacZ activity (first described by Miller, 1972) can be challenging for a large number of samples, is prone to variability, and involves hazardous compounds for lysis (e.g. chloroform or toluene).

Here we describe a single-step assay using a 96-well microplate reader, using a proven alternative cell permeabilisation method. This modified protocol reduces handling time by 90%.

Various β-galactosidase protocols for bacteria have been described, adapting some of the Miller (1972) method steps for use in plate readers (Menzel, 1989; Arvidson, 1991; Bianco, 1994; Griffith and Wolfe, 2002). However, these methods include many of the drawbacks inherent to the original method and remain labour-intensive.

One of the challenges in further speeding up this assay is the cell permeabilisation stage, which is required for the o-nitrophenyl-beta-D-galactopyranoside (ONPG) substrate to enter the cell and interact with β-galactosidase. This typically requires the transfer of cultures due to the fact that permeabilisation is normally performed using chloroform/SDS or toluene (Miller, 1972; Miller 1992), which can interfere with the optical density readings in standard microtitre plates. Deep well nonreactive polypropylene blocks have been suggested (Griffith and Wolfe, 2002), however the organic solvents were reported to be difficult to manipulate using multichannel pipettes (Thibodeau, 2004).

An alternative permeabilisation method was proposed using PopCulture® Reagent (Thibodeau, 2004), a compound used in protein purification. PopCulture® Reagent punctures the cell wall without denaturing soluble proteins or interfering with optical density readings, with the β-galactosidase remaining stable for up to 18 hours (Thibodeau, 2004). The cell lysis efficiency can be further enhanced by the addition of chicken egg white lysozyme, which hydrolyses the peptidoglycan in cell walls (Grabski, 2001). This protocol was shown to produce similar results to the traditional chloroform/SDS method used for cell lysis (Thibodeau, 2004). This approach allowed for kinetic readings rather than endpoint readings, obviating the need for stopping the reaction with Na_2CO_3 and thereby improving accuracy.

While this new permeabilisation method has improved accuracy of the assay and reduced handling time for a large number of samples, the time taken to process smaller numbers of samples remains largely unchanged. Here we describe a streamlined version of these methods to condense the assay from several liquid handling steps into a single-step assay, decreasing the labour intensity irrespective of sample size.

The one-step approach aims to combine i) OD_{600} measurement, ii) cell permeabilisation, iii) ONPG breakdown, and iv) kinetic OD_{420} quantification into a single step. The approach involves transferring 80 µL cells and 120 µL custom Bgal mix (60mM Na_2HPO_4, 40mM NaH_2PO_4, 10mM KCl, 1mM $MgSO_4$, 36mM β-mercaptoethanol, 166µL/ml T7 lysozyme, 1.1mg/ml ONPG, 6.7% PopCulture ® Reagent) to a microtitre plate, followed by kinetic OD_{420} and OD_{600} quantification on a FLUOstar Omega Microplate Reader (BMG LABTECH). These are then converted into Miller Units [MU] using the MARS