Abstract

This method is a colorimetric assay for determining β-galactosidase activity of strains containing a β-galactosidase reporter fusion. β-galactosidase is a hydrolase enzyme that catalyzes the hydrolysis of β-D-galactosides into monosaccharides. In the assay described below, the artificial chromogenic substrate ortho-nitrophenyl-β-D-galactopyranoside (ONPG) is used; when the β-galactosidase cleaves ONPG, ortho-nitrophenol is released. ONPG is colourless, while ortho-nitrophenol has a yellow colour, and absorbs light (λ_{max} = 420 nm). Enzyme activity is measured by the rate of appearance of a yellow colour using a spectrophotometer. Note: To remove endogenous β-galactosidase activity this procedure requires the use of lac^− strains.

Content

Preparation

The following solutions are prepared prior to use:

**Z-buffer pH 7.0:**

- 60 mM Na$_2$HPO$_4$
- 40 mM NaH$_2$PO$_4$
- 10 mM KCl
- 1 mM MgSO$_4$

Bring to the correct pH and then autoclave. Store at room temperature. Add β mercaptoethanol (βME) to 50 mM final concentration as required (270 µl per 100 ml buffer). Z-buffer containing βME cannot be stored.

**ONPG (ortho-nitrophenyl-β-D-galactopyranoside)**

Dissolve freshly each day to a final concentration of 4 mg/ml in Z-buffer containing no β-mercaptoethanol.

**Na$_2$CO$_3$**

Prepare a 1 M solution beforehand.

Isolation of samples for analysis

1. Culture (of a known OD$_{600}$) is eluted from the chemostat and 1 ml promptly spun down for each sample. NB. I usually take a few aliquots for each sample.
2. Centrifuge 10,000 rpm, 5 minutes, room temperature in benchtop microcentrifuge.
3. Decant supernatant and, if necessary, store pellet at -20 °C. If stored then the assay must be carried out the next day.

Carrying out colorimetric assay

4. Thaw cell pellets (if necessary) and resuspend in 1 ml Z-buffer+βME.
5. Measure the OD$_{600}$ of the cell suspension (blank against Z-buffer+βME).
6. Carry out a dilution of culture if required in Z-buffer+βME. See discussion below.
7. Add 100 µl of culture dilution to 700 µl Z-buffer+βME.
8. Add 20 µl chloroform.
9. Add 10 µl 0.1% SDS.
10. Vortex for 10 seconds.
11. Incubate for 5 minutes at 28 °C.
12. Start the reaction by adding 200 µl of pre-warmed 4 mg/ml ONPG in Z-buffer (no BME). Start the timer.
13. Incubate the reactions at 28 °C until they are straw yellow. This should ideally take > 5 min.
14. Stop reaction by adding 500 µl of 1 M NaCO$_3$ and note the time.
15. Centrifuge sample at full speed for 5 minutes in microcentrifuge to remove debris and chloroform.
16. Measure $A_{420}$ against a blank containing Z-buffer, ONPG, CHCl$_3$, SDS,NaCO$_3$. $A_{420}$ should be between 0.6 and 0.9, although readings between 0.1 and 1.2 are acceptable. If too high then repeat with a diluted sample, if too low then incubate longer to give a more intense yellow.
17. Calculate B-gal activity in ‘Miller units’.

$$\text{Activity (Miller units)} = \left( A_{420} \cdot \text{dilution factor} \cdot 1000 \right) / \left( \text{time} \cdot \text{vol} \cdot \text{OD} \right)$$

Activity units are in $A_{420} \text{min}^{-1} \text{mL}^{-1} \text{OD}_{600}^{-1}$ although this is not normally written.

Possible Variations

Some recent papers have indicated that OD$_{600}$ may be an unsuitable reference when assaying cellular material in a β-gal assay when cell size differences are possible. In these circumstances viable cell count may be more applicable, or here, dry-cell-weight be superior. I have not tried these although they may be of relevance.

Parts List

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Changes to Previous Versions

<table>
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No previous versions exist

Discussion and Comments

For assaying the low-copy plasmid FF(-41.5)/RW50 encoding an FNR-activated β-gal reporter (Barnard et al., 2003) the following dilutions were needed

1:10 were necessary for 0 to 80% AAU
1:5 dilutions for 100% AAU.