Protocol

TD-P Revision 3.0

Creation Date: 5/25/2016
Revision Date: 9/5/2018

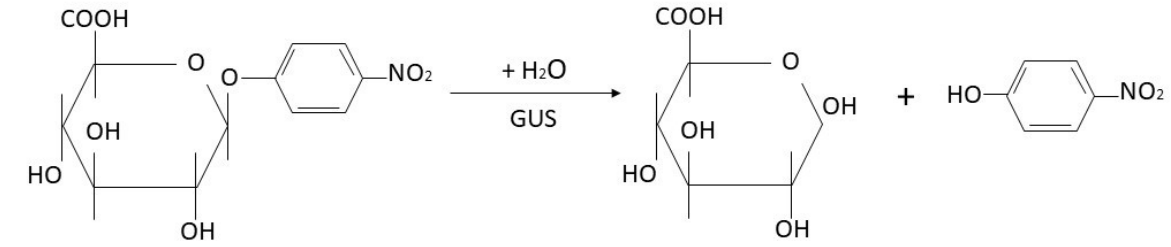
Detection of β-Glucoronidase (GUS)

Utilizing PNPG

Introduction

β-Glucuronidase (GUS) is a lysosomal enzyme isolated from *E. coli* that hydrolizes β-linked D-glucuronides to D-Glucuronic acid and aglycones. GUS has become an important reporter gene and is used in the study of regulation of gene transcription in various organisms. It is often used as a reporter in studies involving transgenic plants because it is not endogenously expressed in plants. One assay often used in detection of GUS is spectrophotometric analysis since p-nitrophenol glucuronide is cleaved by GUS to p-nitrophenol, which is a chromopheric product and has a high absorbance at 405 nm. Detection of the yellow product (p-nitrophenol) indicates successful expression of the *gusA* gene.

Reaction catalyzed by GUS:



P-Nitrophenyl β-D-glucuronide (pNPG)

β-D-glucuronic acid

P-Nitrophenol (yellow color)

Materials

- Purified enzyme
- Phosphate
- β-mercaptoethanol
- PNPG (GoldBio Catalog # N-325)
- Spectrophotometer with absorbance at 405 nm

Storage and Handling

- Store PNPG desiccated at -20°C and protect from light.

Gold Biotechnology
St. Louis, MO
Ph: (800) 248-7609
Web: www.goldbio.com
Email: contactgoldbio86@goldbio.com

- This product may be shipped in blue ice and should be stored immediately upon arrival at -20°C.

Method

This assay should be carried out at 22°C.

1. Suspend purified enzyme in the following buffer:
    a. 20mM (final concentration) phosphate at pH 7.4 and 10mM (final concentration) β-mercaptoethanol. This solution should be prepared fresh every time.

2. Add PNPG to the buffer enzyme solution to a final concentration of 1mM.

3. Monitor absorbance continuously at 405 nm.

Calculations

$$1^{*}unit\ of\ activity = \frac{1\mu mol\ of\ p-nitrophenol}{min}$$

*At a pH of 7.4 the extinction coefficient of p-nitrophenol is approximately 9000 M^{-1} cm^{-1}.

Associated Products

- PNPG (GoldBio Catalog # N-325)

References

Aich, S., Delbaere, L. T., and Chen, R. (2001). Expression and Purification of *Escherichia coli* β-Glucuronidase. *Protein expression and purification*, 22(1), 75-8.

Gallagher, S. (1992). GUS Protocols: Using the GUS gene as a reporter of gene expression. Elsevier. Doi:10.1016/C2009-0-03175-4.