# Protocol

TD-P Revision 3.0

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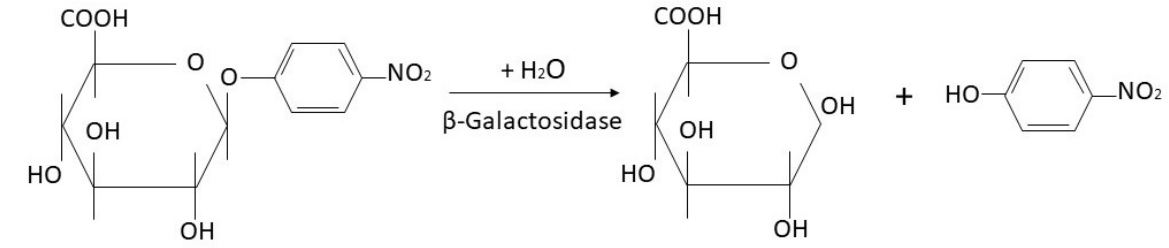
# β-Gal Enzyme Assay
## utilizing PNPG

## Introduction

β-galactosidase has various enzymatic activities that have been exploited to study gene expression regulation and many other scientific processes. The natural substrate for β-galactosidase is lactose. However, this enzyme can hydrolyze 4-Nitrophenyl-β-D-glucuronic acid (PNPG) into D-galactose and p-nitrophenol, which can be measured by determining the absorbance at 420 nm at pH 7. This assay is specifically useful in enzyme kinetic studies. Here, we present a protocol to determine β-galactosidase activity using PNPG as a substrate.

Hydrolysis of PNPG by β-galactosidase:




P-Nitrophenyl β-D-glucuronide (pNPG)

D-galactose

P-Nitrophenol (yellow color)

## Materials

- Microtiter plate
- 20mM Tris-HCl and 0.6mM $CaCl_2$ buffers
- PNPG (GoldBio Catalog # N-325)
- Eppendorf tubes
- Spectrophotometer

## Storage and Handling

- Store PNPG desiccated at -20°C. Protect from light.
- This product may be shipped on blue ice and should be stored immediately upon arrival at -20°C.

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## Method

1. Prepare an assay buffer of 20mM Tris-HCl and 0.6mM $CaCl_2$ with a pH of 8.0, for a final concentration of 10mM.

2. Mix sample and assay buffer for a final volume of 110 µl and place in ice.

3. Add an equivalent amount of 10mM PNPG to each tube and then vortex.

4. Remove 100 µl from the primary tube and add to duplicate wells.

5. Incubate at 37°C for 1.5 hours in microtiter plates.

6. Measure absorbance at 405 nm to measure release of *p*-nitrophenyl and compare to standard curve.

## Associated Products

- PNPG (GoldBio Catalog # N-325)

## References

Fisher K.L. and Woods J.P. (2000). Determination of β-glucosidase enzymatic function of the Histoplasma capsulatum H antigen using a native expression system. Gene, 247 191-197.

Juers, D. H., Matthews, B. W., and Huber, R. E. (2012). LacZ β-galactosidase: Structure and function of an enzyme of historical and molecular biological importance. *Protein Science,* 21(12), 1792-1807. Doi:10.1002/pro.2165.