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# **ProductInformation**

# 2-Nitrophenyl b-D-galactopyranoside

Product Number **N1127** Storage Temperature -20 °C

### **Product Description**

Molecular Formula:  $C_{12}H_{15}NO_8$ Molecular Weight: 301.3 CAS Number: 369-07-3 Melting Point: 193-194 °C <sup>1</sup> Specific Rotation: -67° (1 g/100 ml water at 25 °C) Synonym: ONPG

This product (ONPG) is a colorimetric substrate for  $\beta$ -galactosidase. This product is hydrolyzed to o-nitrophenol by the enzyme. o-Nitrophenol is yellow in alkaline solution and can be quantitated at 410-420 nm.<sup>2</sup> The molar extinction coefficient is 2.13 x 10<sup>4</sup> at pH 10.2.<sup>3</sup> We have used absorbance readings at 410 nm in the determination of the activity of  $\beta$ -galactosidase using this product as the substrate. The K<sub>m</sub> for  $\beta$ -galactosidase (from *E. coli*) is 9.5 x 10<sup>-4</sup> M in Tris buffer, pH 7.6, at 20 °C.<sup>4</sup> The V<sub>max</sub> for  $\beta$ -galactosidase for this substrate is 134 µmoles/min/mg protein.<sup>5</sup>

## **Precautions and Disclaimer**

For Laboratory Use Only. Not for drug, household or other uses.

#### **Preparation Instructions**

A stock solution of this product can be prepared in deionized water at a concentration of 3 mg/ml.

Alternatively, a stock solution may be prepared in phosphate buffer. When used as a substrate for  $\beta$ -galactosidase, a solution of approximately 20.5 mg/ml is prepared in 100 mM sodium phosphate buffer, pH 7.3. The solution is gently warmed to completely dissolve the product.

#### References

- 1. J. Am. Chem. Soc., 75, 1758 (1953).
- Molecular Cloning: A Laboratory Manual, 2nd ed., 3, Sambrook, J., et al., Cold Spring Harbor Laboratory (1989), p. 1666.
- 3. Adv. Carbohydr. Chem., 16, 239 (1961).
- 4. Enzyme Handbook, **II**, Barman, T. E., Springer-Verlag (Berlin-Heidelberg: 1969), p. 581.
- 5. Wallenfels, K., β-Galactosidase (Crystalline). Methods in Enzymology, **5**, 212-218 (1962).

AJH/VLE 8/08

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