

Specification

2-Nitrophenyl-β-D-Galactopyranoside *BioChemica*

A1272

Physical Description:	Solid
Product Code:	A1272
Product Name:	2-Nitrophenyl-β-D-Galactopyranoside <i>BioChemica</i>
Specifications:	Assay (HPLC): min. 99 % α 20°C/D; 1 %, H ₂ O: -67° - -71° 2-Nitrophenol: max. 0.03 %
WGK:	1
Storage:	-20°C protected from light
Molecular Formula:	C ₁₂ H ₁₅ NO ₈
M:	301.26 g/mol
CAS:	369-07-3
EINECS:	206-716-1
CS:	29389090
Comment	<p>ONPG is one of the major substrates in β-galactosidase enzyme assays. The properties of this enzyme have been studied in detail (e. g. ref. 1-3). Expression plasmids with the coding sequence of β-Galactosidase are widely used as internal controls for transfection experiments in expression and transcription studies, usually under the control of a strong enhancer (SV40, CMV, RSV, HIV etc.). The enzyme activity is easily determined from cell extracts (e. g. ref. 4). The cleavage of ONPG releases the yellow dye 4-nitrophenyl and absorption is measured at 405 - 420 nm by spectrophotometry. A commonly used reaction buffer is: 60 mM Na₂HPO₄ / 39 mM NaH₂PO₄, pH 7.0 / 10 mM KCl / 1 mM MgSO₄ / 2 mM DTT and 1 mg/ml ONPG. ONPG is of low solubility. The solid ONPG is added to the reaction buffer and dissolved by warming the buffer to 37°C and/or well mixing. DTT is added freshly (like ONPG) from a 1 M stock solution (4). Note: For the investigations mentioned above, the bacterial β-galactosidase (<i>E. coli</i>) is used. The pH-optimum of this enzyme is pH 7.3. In mammalian cells exists a lysosomal β-galactosidase with a pH-optimum at 3.5. Make sure, that the pH of the reaction buffer is correct, otherwise there could be a significant background by the endogenous enzyme!</p>

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Bibliography

(1)Wallenfels, K. *et al.* (1960) *Biochem. Z.* **333**, 377-394Untersuchungen über milchzuckerspaltende Enzyme. (2)Wallenfels, K. (1962) *Methods Enzymol.* **5**, 212-219 β -Galactosidase\): Assay methods. (3)Levy, G.A. & Conchie, J. (1966) *Methods Enzymol.* **8**, 571-584Mammalian glycosidases and their inhibition by Aldonolactones. (4)Janknecht, R. *et al.* (1995) *Oncogene* **10**, 1209-1216SAP1a is a nuclear target of signaling cascades involving ERKs.

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