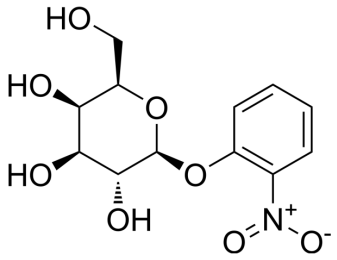
ONPG

Cat. No.:	HY-15926
CAS No.:	369-07-3
Molecular Formula:	$C_{12}H_{15}NO_8$
Molecular Weight:	301.25
Target:	Biochemical Assay Reagents
Pathway:	Others
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



SOLVENT & SOLUBILITY

In Vitro

H_2O : 8.33 mg/mL (27.65 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	3.3195 mL	16.5975 mL	33.1950 mL
	5 mM	0.6639 mL	3.3195 mL	6.6390 mL
	10 mM	0.3320 mL	1.6598 mL	3.3195 mL

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

ONPG is a colorimetric and spectrophotometric substrate for detection of β-galactosidase activity.

In Vitro

The enzyme displays high hydrolysis ability for ONPG (100%) and moderate activity for its natural substrate lactose (25.7%). However, the hydrolysis ability of the enzyme towards all other chromogenic nitrophenyl analogues is very weak, indicating that Gal308 is a β-galactosidase with narrow substrate specificity. To investigate the kinetic parameters of recombinant enzyme, the Michaelis-Menten constants (K_m), turnover numbers (k_{cat}), and catalytic efficiencies (k_{cat}/K_m) of Gal308 for ONPG and lactose are determined. The k_{cat} and K_m values are 464.7±7.8 s^{-1} and 2.7±0.3 mM for ONPG, and 264.2±2.1 s^{-1} and 7.1±0.8 mM for lactose, respectively. The k_{cat}/K_m value of the enzyme for ONPG (172.1 $s^{-1}mM^{-1}$) is 4.6-fold higher than that for lactose (37.2 $s^{-1}mM^{-1}$), which clearly demonstrated that the catalytic efficiency of Gal308 for ONPG is much higher than that for lactose[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	The β-galactosidase activity is measured using two substrates including ONPG and lactose in this study. The β-galactosidase activity for ONPG is measured by following the amount o-nitrophenol released from ONPG. The reaction mixture is composed of 100 μL of the enzyme solution and 400 μL of ONPG solution (2.5 g/L in 100 mM Tris-HCl buffer at pH 6.8). After incubation at 78°C for 15 min, the reaction is terminated by adding an equal volume of 1 M Na_2CO_3. The released o-nitrophenol is quantitatively determined by measuring at A_{405}. One unit of activity is defined as the amount of enzyme needed to produce 1 μmol of o-nitrophenol per minute under the assay condition. The specific activity is expressed as units per milligram of protein. Assays for activity towards lactose are performed in the same buffer containing 100 μL of enzyme solution and 5% lactose, and the reaction is stopped by boiling for 10 min, and the concentration of glucose is determined using a glucose oxidase-peroxidase assay kit. The released glucose is quantitatively determined by measuring A_{492}. One unit of enzyme activity is defined as the amount of activity required to release 1 μmol of glucose per minute[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Cell Res. 2021 Jun;31(6):703-712.
- Biomaterials. 2023 Apr 11, 122122.
- Nano Lett. 2020 May 13;20(5):3602-3610.

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REFERENCES

[1]. Zhang X, et al. Metagenomic approach for the isolation of a thermostable β-galactosidase with high tolerance of galactose and glucose from soil samples of Turpan Basin. BMC Microbiol. 2013 Oct 24;13:237. doi: 10.1186/1471-2180-13-237.

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