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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

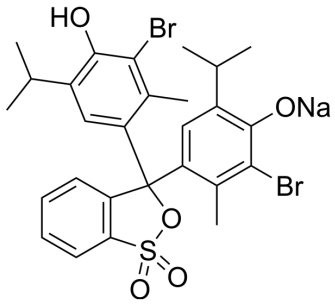
mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Bromothymol Blue sodium

Cat. No.:	HY-D0012A
CAS No.:	34722-90-2
Molecular Formula:	$C_{27}H_{27}Br_2NaO_5S$
Molecular Weight:	646.36
Target:	Others
Pathway:	Others
Storage:	Please store the product under the recommended conditions in the Certificate of Analysis.



BIOLOGICAL ACTIVITY

Description	Bromothymol Blue sodium salt is a pH indicator. Storage: protect from light.
In Vitro	A first characterization and comparison is done by developing an easy and direct measurement method based on a pH indicator system using Bromothymol Blue (BTB) as the indicator and potassium phosphate as the buffer. A pH-shift assay is developed on the basis of Bromothymol Blue as the pH indicator and potassium phosphate as the buffer component[1]. Three pH indicators are tested for the direct determination of 2- 2-keto-L-gulonic acid (2-KLG) production on a plate. The results show that Bromothymol Blue is superior to the other two indicators in terms of the obvious color change and a suitable pH range (blue to yellow at pH 6.5-7.5). Upon the addition of a Bromothymol Blue solution (0.1%, w/v) to an agar plate, zones surrounding colonies of K. vulgare 07 mutants change their color from blue to yellow because K. vulgare 07 mutants release 2-KLG on agar plates, thereby acidifying surrounding areas around colonies[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	For all three enzymes, an expression in the 96-deep well scale is performed. Therefore, electrocompetent E. coli ArcticExpress (DE3) cells are transformed with the corresponding plasmid. Single clones are picked using the CP7200 Colony Picker and transferred to 96-deep well plates filled with 1.2 mL autoinduction media by a MicroFlo Select dispenser. After incubation (36 h, 37°C at 1,000 rpm), further processing is done manually. First, 100 µL of cell culture is transferred into a 96-well plate (U-shaped bottom) and harvested by centrifugation (4,570 rpm, 10 min at RT) while the supernatant is discarded. The frozen pellets (1 h at -20°C) are thawed at room temperature for one hour to improve cell lysis. Lysis is continued by the addition of 30 µL lysis buffer (3 h, 1,000 rpm, 37°C) containing 2 mM KP_i, pH 7.0, 2 mM $MgCl_2$, 10 µg/mL DNaseI, 100 µg/mL lysozyme. Next, 120 µL buffer (2 mM KP_i, pH 7.0) is added followed by centrifugation (3,000 rpm, 15 min at RT). For the photometric measurement, 20 µL of the crude extract is transferred to a 96-well plate (F-shaped bottom) and the reaction is started by adding 180 µL master mix to give a final volume of 200 µL (2.5 mM KP_i, pH 7.0, 2 mM $MgCl_2$, 25 µg/mL Bromothymol Blue (BTB) and 5 mM keto-deoxy-D-glucarate). The measurements are carried out for 60 min at 2-min intervals. Depending on the enzyme, different time windows are used for the activity calculation[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Pick A, et al. Identification and characterization of two new 5-keto-4-deoxy-D-Glucarate Dehydratases/Decarboxylases. BMC Biotechnol. 2016 Nov 17;16(1):80.

[2]. Yang W, et al. A plate method for rapid screening of Ketogulonicigenium vulgare mutants for enhanced 2-keto-l-gulonic acid production. Braz J Microbiol. 2017 Jul - Sep;48(3):397-402.

Tel: 609-228-6898 Fax: 609-228-5909 E-mail: tech@MedChemExpress.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA