

5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt monohydrate, X-Gluc

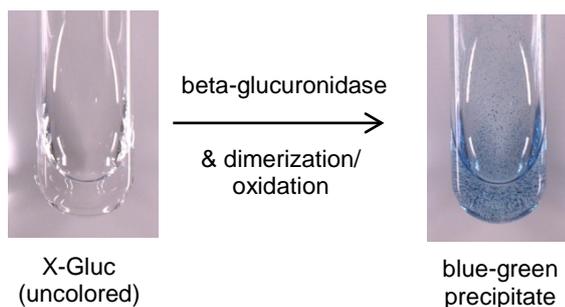
Cat. No. B-7300

M.W. 539.81 g/mol

Introduction

5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt monohydrate (X-beta-D-glucuronide, X-Gluc) is a chromogenic indicator for beta-glucuronidase activity. The enzyme is not widely distributed in bacteria, however almost all *Escherichia coli* strains produce beta-glucuronidase, facilitating specific detection of this species as indication of fecal contamination. The colorless product B-7300 is cleaved by *Escherichia coli* in liquid media or on agar plates and yields a blue-green color/precipitate.

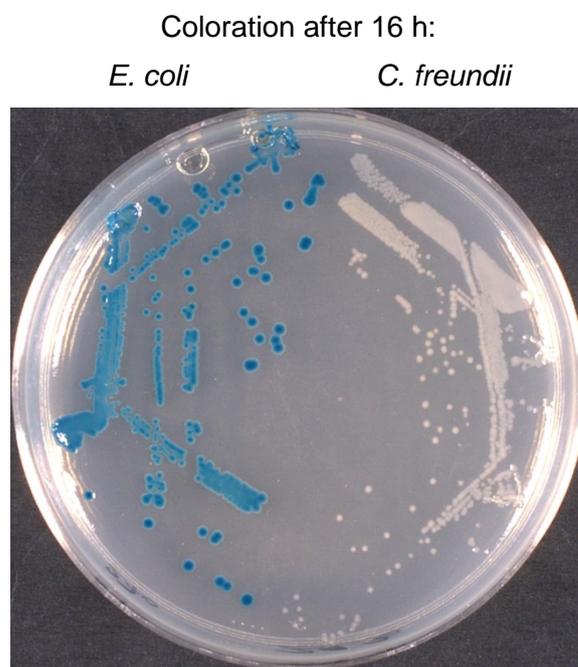
X-Gluc can also be used as detecting agent for beta-glucuronidase activity in reporter gene assays or for identification of positive transformants, which is of particular value in plant science (GUS staining).



Color formation is due to dimerization and oxidation of 5-bromo-4-chloro-3-hydroxyindole which results from enzymatic cleavage of X-Gluc. The reaction proceeds only in the presence of oxygen, i.e., in aerobic cultures. Well detectable colored bacterial colonies or liquid cultures are obtained within 16-48 h. In the presence of purified beta-galactosidase, color develops within minutes to few hours.

Application example

Overnight cultures of *Escherichia coli* ATCC 25922 (beta-glucuronidase positive) and *Citrobacter freundii* ATCC 8454 was diluted to an optical density (600 nm) of 0.1 in sterile saline and streaked out on Nutrient Agar containing 0.2 mM X-Gluc and 0.5 mM of the inducer 1-O-Methyl-beta-D-glucuronic acid, sodium salt. The plates were incubated at 37°C for 22 h.



Technical information

Instructions for use:

Add 5-Bromo-4-chloro-3-indoxyl-beta-D-glucuronic acid, cyclohexylammonium salt monohydrate (X-Gluc) as concentrated stock solution in organic solvent after autoclaving

Solubility:

≥ 100 mM (54 mg/mL) in dimethyl sulfoxide

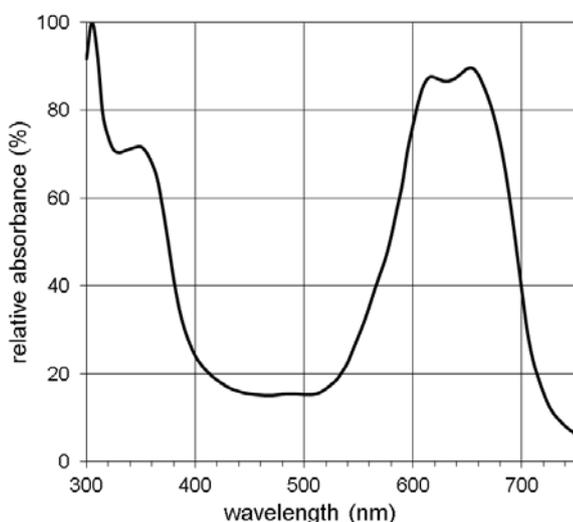
≥ 100 mM (544 mg/mL) in dimethyl formamide

Recommended working concentration: 0.1-0.3 mM (1-3 mL of 100 mM stock solution per L)

For best results also add 0.1-1 mM of the inducer 1-O-Methyl-beta-D-glucuronic acid, sodium salt (Biosynth Cat No. M-3600) to growth media

Absorbance spectrum:

The colored precipitate resulting from enzymatic transformation was solubilized by adding 50% v/v ethanol.



Reference strains:

Escherichia coli ATCC 25922, positive in aerobic culture

Citrobacter freundii ATCC 8454, negative in aerobic culture

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2017-11-28