

Product Information

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5-Bromo-4-chloro-3-indolyl β -D-glucuronide, cyclohexylammonium salt, tablets

Catalog Number **B8049**

Storage Temperature $-20\text{ }^{\circ}\text{C}$

CAS RN 114162-64-0

Synonyms: X-GlcA; BC-Indicator; X-glucuronide

Product Description

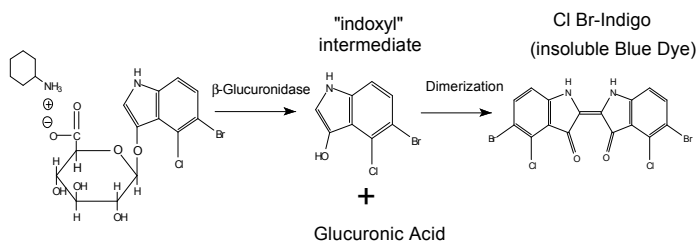
Molecular Formula: $\text{C}_{14}\text{H}_{12}\text{BrClNO}_7 \cdot \text{C}_6\text{H}_{13}\text{N}$

Formula Weight: 521.79

The *gus* operon in *Escherichia coli* is composed of three genes.¹ The first gene, *uidA* (*gusA*), encodes the enzyme β -glucuronidase (GUS). *GusB* encodes a glucuronide permease. The function of the *gusC* gene product is unknown. 5-bromo-4-chloro-3-indolyl β -D-glucuronide (X-GlcA, X-Gluc) has been shown to be a good substrate for GUS, yielding a dark-blue insoluble cleavage product. The reaction (see Figure 1) initially yields a monomeric intermediate, which rapidly oxidizes to form the dimer, dichlorodibromoindigo (ClBr-indigo).

Figure 1.

Hydrolysis of X-GlcA by β -Glucuronidase



The intense coloration and insolubility of ClBr-indigo is ideal for use as an indicator of GUS activity *in situ*. It has been used as an indicator of *E. coli* contamination in various food items² and as an agent in urinary tract infections.³ The *gusA* gene has been used as an indicator of transfection and as a reporter gene for the function of regulatory elements in plants.^{4,5}

If using a known strain of *E. coli* as a positive control for GUS activity, it is important to realize that K-12 strains of *E. coli* contain a defective permease.¹ Even though X-GlcA is an excellent inducer of *uidA* in *E. coli*, K-12 strains require much higher levels of X-GlcA than wild-type strains. With a defective permease, high extracellular levels of X-GlcA are needed to develop sufficient intracellular levels so that *uidA* is adequately induced. In addition, once *uidA* is induced and GUS activity is high, high extracellular levels of X-GlcA are also needed to develop sufficient intracellular levels to react and to yield a dark coloration.

Each tablet is ~ 40 mg with 10 mg of substrate.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To prepare a concentrated stock solution, 1 tablet will dissolve in 100 μl of *N,N*-dimethylformamide (DMF, Catalog Number D4551) with a final volume of ~ 125 μl (80 mg/ml final concentration), or 1 tablet will dissolve in 180 μl of dimethyl sulfoxide (DMSO) with a final volume of 200 μl (50 mg/ml final concentration). These stock solutions can be frozen at -20 or $-70\text{ }^{\circ}\text{C}$.

Storage/Stability

Store the tablets at $-20\text{ }^{\circ}\text{C}$. When stored at $-20\text{ }^{\circ}\text{C}$, the tablets are stable for at least one year. Tablets are good as long as color remains white.

Procedure

As an indicator for the presence of *E. coli* in natural materials

Sigma's functional test procedure is as follows: Prepare LB Agar (Catalog Number L2897) or LB Agar EZMix™ Powder (Catalog Number L7533). Cool to 55 °C. Add 250 µl of a 40 mg/ml stock solution of B8049 in DMSO to 100 ml of LB agar and mix gently to dissolve. The final concentration of X-GlcA in the medium will be 100 µg/ml. Pour plates and allow to cool for a few hours or overnight. Streak one plate with a *uidA*⁺ strain of *E. coli* (ATCC 11303) and a second plate with a *uidA*⁻ strain of *E. coli* (GMS407). Incubate the plates at 37 °C for 24 hours.

As a substrate for the GUS reporter system to study plant gene expression

Please refer to published procedures.¹

References

1. Gallagher, S.R., Gus Protocols: Using the GUS Gene as a Reporter of Gene Expression. Academic Press, Inc. (San Diego, CA: 1992).
2. Delisle, G.J., and Ley, A., Rapid Detection of *Escherichia coli* in Urine Samples by a New Chromogenic β-Glucuronidase Assay. J. Clin. Microbiol., **27**,778-779 (1989).
3. Restaino, L., *et al.*, Use of the Chromogenic Substrate 5-Bromo-4-Chloro-3-Indolyl β-D-Glucuronide (X-Gluc) for Enumerating *Escherichia coli* in 24 h from Ground Beef. J. Food. Prot., **53**, 508-510 (1990).
4. Bomineni, V.R., *et al.*, Transformation of white spruce (*Picea glauca*) somatic embryos by microprojectile bombardment. Plant Cell Reports, **13**, 17-23 (1993).
5. Ellis, D.D., *et al.*, Stable Transformation of *Picea glauca* by Particle Acceleration. Biotechnology, **11**, 84-89 (1993).

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