Product Information

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

Tablet

B8049

Product Description

Synonyms: X-GlcA; BC-Indicator; X-glucuronide (5-Bromo-4-chloro-3-indolyl β-D-glucuronide component)

CAS Registry Number: 114162-64-0
(X-GlcA component)

Molecular Formula: $C_{14}H_{12}BrClNO_7 \bullet C_6H_{13}N$
(X-GlcA component)

Molecular Weight: 521.79 (X-GlcA component)

The gus operon in *Escherichia coli* is composed of three genes:[1]

- *uidA* (*gusA*), which encodes β-glucuronidase (GUS).
- *gusB*, which encodes a glucuronide permease
- *gusC*, which encodes a 44 kDa protein located in the outer membrane of *E. coli*, with an as-yet undetermined function[2]

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 dichloro-dibromoindigo (Cl Br-indigo).

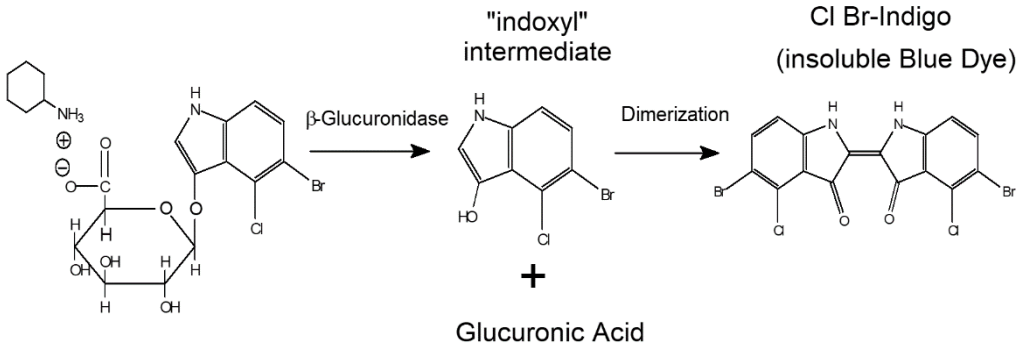
The intense coloration and insolubility of Cl Br-indigo is ideal for use as an indicator of GUS activity *in situ*. Cl Br-indigo has been used as an indicator of *E. coli* contamination in various food items[3] and as an agent in urinary tract infections.[4] The *gusA* gene has been used as an indicator of transfection and as a reporter gene for the function of regulatory elements in plants.[5,6]

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.

Reagent

Each tablet is ~40 mg and contains 10 mg of X-GlcA substrate.

Figure 1. Hydrolysis of X-GlcA by β-Glucuronidase



Precautions and Disclaimer

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

Storage/Stability

Store the tablets at –20 °C. When stored at –20 °C, the tablets are stable for at least one year. Tablets are good as long as their color remains white.

Preparation Instructions

To prepare a concentrated stock solution, dissolve one tablet either in:

- 100 µL of *N,N*-dimethylformamide (DMF) with a final volume of ~125 µL (80 mg/mL final concentration)
- 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 either –20 °C or –70 °C.

Procedure

As an indicator for the presence of *E. coli* in natural materials, one functional test procedure is as follows:

1. Prepare LB Agar (Cat. No. L2897) or LB Agar EZMix™ Powder (Cat. No. L7533).
2. Cool to 55 °C.
3. Add 250 µL of a 40 mg/mL stock solution of Cat. No. B8049 in DMSO to 100 mL of LB agar. Mix gently to dissolve. The final concentration of X-GlcA in the medium will be 100 µg/mL.
4. Pour plates. Allow to cool for a few hours or overnight.
5. Streak one plate with a *uidA*$^{+}$ strain of *E. coli* (ATCC 11303) and a second plate with a *uidA*$^{-}$ strain of *E. coli* (GMS407).
6. Incubate the plates at 37 °C for 24 hours.

As a substrate for the GUS reporter system to study plant gene expression, various published procedures are available.[1]

References

1. Gallagher, S.R. (ed.), *GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression*. Academic Press, Inc. (San Diego, CA: 1992).
2. Liang, W.-J. *et al*., *J. Bacteriol*., **187(7)**, 2377-2385 (2005).
3. Delisle, G.J., and Ley, A., *J. Clin. Microbiol*., **27(4)**, 778-779 (1989).
4. Restaino, L. *et al*., *J. Food. Prot*., **53(6)**, 508-510 (1990).
5. Bomineni, V.R. *et al*., *Plant Cell Rep*., **13(1)**, 17-23 (1993).
6. Ellis, D.D. *et al*., *Bio/Technology*, **11**, 84-89 (1993).

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