Product Information

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

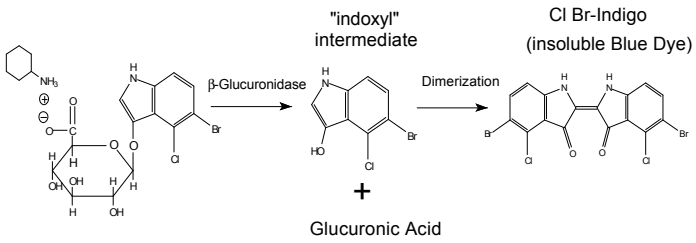
Catalog Number **B8174**
Storage Temperature –20 °C

CAS RN 129541-41-9
Synonyms: X-GlcA; BC-Indicator; X-glucuronide

Product Description
Molecular Formula: $C_{14}H_{12}BrClNO_7 \cdot Na$
Formula Weight: 444.59

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 items[2] 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 water with a final volume of ~125 µl (80 mg/ml final concentration). This can be frozen at –20 or –70 °C. The tablet may also be diluted to the final concentration for use by dissolving directly into a reaction buffer solution or bacteriological growth medium, depending on the desired application.

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 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 one 10 mg tablet per 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]

Results

The *uidA*$^+$ cells produced dark-blue colonies indicating the expression of the β-glucuronidase gene and the *uidA*$^-$ cells produced non-colored colonies indicating the absence of expression.

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 *Picia glauca* by Particle Acceleration. Biotechnology, **11**, 84-89 (1993).

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