

### **Product Name & Product No.**

Product Name: X-GlcA 【129541-41-9】  
Product No.: X003

### **Spec.**

Enzymatic Colorimetric Reagent, For GUS  
Detection  
5-Bromo-4-chloro-3-indolyl- $\beta$ -D-Glucuronic  
Acid, Sodium Salt  
C<sub>14</sub>H<sub>12</sub>BrClNO<sub>7</sub>Na  
M.W.: 444.6  
Assay: 98.0% min.  
Solubility(2% Soln. in DMF): Clear and  
Colorless

### **Description**

X-GlcA, 5-Bromo-4-chloro-3-indolyl- $\beta$ -D-Glucuronic acid is a substrate for  $\beta$ -D-Glucuronidase (GUS) encoded by the *gusA* gene. The substrate is used as a qualitative histochemical marker of specific GUS expression in cells and tissue. X-GlcA is cleaved by GUS at the  $\beta$ 1 glucuronic bond between glucuronic acid and the 5-Bromo-4-chloro-3-indolyl part of X-GlcA via hydrolysis. The cleavage of X-GlcA results in the precipitation of water insoluble blue dichloro-dibromo-indigo precipitate at the site of enzymatic cleavage. Colour formation requires three separate reactions. After enzymatic turnover, the released indoxyl derivative dimerizes and is subsequently oxidized to the final indigo dye.

### **Preparation Instruction**

1% (w/v) X-GlcA, sodium salt in sterile N, N-dimethylformamide or deionized water (10 mg/mL).

### **Note:**

X-GlcA, sodium salt is unstable in water. Use immediately after dissolution. X-GlcA solution should be added to media after it is cooled below 55 °C.

### **Applications**

1. Suitability for *uidA* (GUS) Detection in *Escherichia coli*  
*uidA*<sup>-</sup> and *uidA*<sup>+</sup> *Escherichia coli* cells were streaked on separate Luria plates containing 1% (w/v) peptone, 0.5% (w/v) yeast extract, 0.5% (w/v) NaCl, 0.7% (w/v) agar and 0.33 mg/mL X-GlcA, sodium salt. The plates were incubated overnight at 37 °C. After 24-48 hours at 37 °C, the *uidA*<sup>+</sup> cells produced blue colonies indicating the expression of the  $\beta$ -glucuronidase gene and the *uidA*<sup>-</sup> cells produced white colonies indicating the absence of expression.
2. Suitability as Substrate for  $\beta$ -Glucuronidase  
A qualitative enzymatic assay was run in 0.1 M acetate buffer, pH 5.0 at 37 °C and 0.1 mg/mL X-GlcA with 6,000 units of  $\beta$ -glucuronidase in a 3 mL reaction mix. Suitability is determined by measuring the change in absorbance at 400 nm in one minute. A positive rate represents suitability.

### **Storage**

Store at 4°C; Protect from light and humidity.  
For long term storage hold at -20°C.