Certificate of Analysis

PRODUCT: 5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-GLUC) (cyclohexylammonium salt)

CATALOG No. 631721

SIZE 100 mg

LOT NUMBER:
Specified on product label

STORAGE CONDITIONS
Store dry at –20°C.

SHORT TERM STORAGE
Make a 20 mg/ml solution of X-GLUC in DMSO. This solution can be stored at –20°C for at least 1 month.

SHELF LIFE
1 year from date of receipt under proper storage conditions.

SHIPPING CONDITIONS
Dry ice (–70°C)

DESCRIPTION
Substrate for β-glucuronidase, an acid hydrolase encoded by the *gusA* gene (1). When used in histochemical assay methods, X-GLUC will give a blue precipitate in the presence of β-glucuronidase.

FORM
white crystalline solid

MOLECULAR WEIGHT: 522

MELTING POINT: 236.8°C

FOR RESEARCH USE ONLY

QUALITY CONTROL DATA

1. A homogenous spot was produced by silica gel thin-layer chromatography.
2. The structure was confirmed by infra-red spectrum analysis.

REFERENCES

1. Jefferson, R. A. (1987) Assaying chimeric genes in plants: The GUS gene fusion system. *Plant Mol. Biol. Rep.* **5**(4):387–405.
2. Jefferson, R. A., Kavanaugh, T. A. & Bevan, M. W. (1987) GUS Fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**(13):3901–3907.

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PA124316)

Histochemical Assay

The best substrate for histochemical localization of β-glucuronidase activity in tissues and cells is 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc). This substrate works very well, giving a blue precipitate at the site of enzyme activity. There are numerous variables that affect the quality of the histochemical localization. It is worthwhile understanding the nature of the reaction to better eliminate the variables. The product of glucuronidase action on X-Gluc is not colored. Instead, the indoxyl derivative produced must undergo an oxidative dimerization to form the insoluble and highly colored indigo dye. This dimerization is stimulated by atmospheric oxygen, and can be greatly enhanced by using an oxidation catalyst such as a K^+ ferricyanide/ferrocyanide mixture (12). Without such a catalyst, the results are often very good, but one must be concerned about the possibility that localized peroxidases may enhance the apparent localization of glucuronidase. One will not get false positives, but the relative degree of staining may not necessarily reflect the concentrations of glucuronidase.

Fixation conditions will vary with the tissue and its permeability to the fixative. Glutaraldehyde does not easily penetrate leaf cuticle, but is immediately available to stem cross sections. Fixation with 2.5% glutaraldehyde in 0.1 M $NaPO_4$, pH 7.0 for 2–3 minutes on ice seems to leave a reasonable amount of GUS activity, when followed by extensive washing. One should test fixation empirically in any new system. Formaldehyde seems to be a more gentle fixative than glutaraldehyde, and can be used for slightly longer times.

X-Gluc can be used at concentrations from 1–2 mM substrate in phosphate buffer. Incubation is at 37°C for anywhere from a few minutes to overnight. The quality of the localization does not decay terribly with lengthy assays. The use of an oxidation catalyst is recommended, for instance 0.5 mM potassium ferricyanide, 0.5 mM potassium ferrocyanide. The enzyme is moderately inhibited by the catalyst, but localization of the product is sometimes enhanced. One can include 10 mM EDTA in the incubation mix to somewhat mitigate the inhibition by the catalyst.

An alternative histochemical assay for GUS uses Naphthol ASBI-glucuronide coupled to a diazo dye. Incubate the formaldehyde or glutaraldehyde fixed tissue or whole mounts in 0.1 M $NaPO_4$, pH 7.0, with 1 mM Napthol ASBI glucuronide in a moist chamber at 37°C. For very low amounts of the enzyme lengthy incubation may be necessary, but gives poor localization of activity due to diffusion of the product. The specimen is then washed in phosphate buffer and coupled using a fresh solution of diazotized dye in phosphate buffer. Post-coupling with a 1–5 mg/ml solution of Fast Garnet GBC in phosphate buffer, pH 7, gives a very nice result after as little as 30 seconds coupling.

Notice to Purchaser

5-Bromo-4-chloro-3-indolyl-β-D-glucuronic acid (X-GLUC) (cyclohexylammonium salt)

CATALOG NO.
631721

NOTICE TO PURCHASER:
Clontech products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Clontech products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with the licensing requirements listed below and described on the product's web page at http://www.clontech.com. It is your responsibility to review, understand and adhere to any restrictions imposed by these statements.

LICENSING STATEMENTS:

This document has been reviewed and approved by the Clontech Quality Assurance Department.

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: **tech@clontech.com**

United States/Canada	**Asia Pacific**	**Europe**	**Japan**
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116