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Product Information

5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside
tablet

Catalog Number **B6024**
Storage Temperature –20 °C

CAS RN 7240-90-6
Synonyms: BCIG, X-Gal, 5-Bromo-4-chloro-3-indolyl β-D-galactoside

Product Description

Molecular Formula: $C_{14}H_{15}BrClNO_6$
Molecular Mass: 408.63

5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside, commonly known as X-Gal, is a histochemical substrate for β-galactosidase.[1] X-Gal is cleaved by β-galactosidase to yield an insoluble blue precipitate.[1] X-Gal is particularly useful in molecular biology applications to detect the activity of β-galactosidase, which is frequently used as a reporter gene.[2-4]

In cloning, X-Gal is used to detect insertion of foreign DNA into the lacZ region of plasmid DNA using α-complementation, which is based on vectors such as the pUC and the M13mp series that carry a fragment of the β-galactosidase gene encoding an α-fragment of β-galactosidase.[5] Insertion of DNA into the lacZ region results in the loss of β-galactosidase activity. Lac+ bacterial colonies resulting from α-complementation will appear blue. Bacterial colonies containing plasmid with DNA inserted in the lacZ region will be incapable of α-complementation and will appear white.[5]

Many other applications also use X-Gal as a substrate to detect β-galactosidase activity. These include β-galactosidase-antibody linked immunoassays and immunohistochemistry,[6-9] coliphage detection based on β-galactosidase induction,[10] and the detection of micrometastasis formation during tumor progession.[4]

This product is in tablet form, with 5 mg substrate per tablet. X-gal is available in several other forms. Catalog Numbers B4252 and B9146 are in powder form. The Molecular Biology Reagent products, B9146 and B6024, are quality control-tested for identification of lac+ bacterial colonies. (See also the Related Products section.)

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Prepare a 20 mg/mL stock solution of X-Gal in *N*,*N*-dimethylformamide (DMF) or dimethylsulfoxide (DMSO).[11,12] Sterilization is not required.[3] Store stock solutions in glass containers protected from light at –20 °C.[13] Solutions may be stored at –20 °C for 6–12 months. If a solution turns pink, it should be discarded.

Storage/Stability

Store at –20 °C protected from light.

Procedures

Blue/White Colony Screening

A. X-Gal included in agar:
   1. Add 5 mL of X-Gal stock solution and 5 mL of 0.1 M isopropyl-β-D-thiogalactoside (IPTG, e.g., Catalog Number I6758) for each 1 liter of autoclaved medium agar (e.g. LB agar, e.g. Catalog Number L2897) containing appropriate antibiotics just prior to pouring.[14] The medium should be below 55 °C.
   2. Plate cells on cooled agar.
   3. Incubate overnight at 37 °C.

B. X-Gal applied to top of agar:
   1. To a premade LB agar plate (e.g., prepared using LB agar, e.g. Catalog Number L2897), add 40 µL of X-Gal stock solution (at room temperature) and 4 µL of a 200 mg/mL solution of IPTG.[5]
   2. Spread solution over the entire surface of the plate.
   3. Incubate at 37 °C until the fluid is no longer visible. This may take several hours.
   4. Plate cells and incubate overnight at 37 °C. Using X-Gal only on the surface rather than throughout the agar plates may help minimize costs.[5]

Immunocytochemistry[2,3,4,9]

1. Prepare X-Gal Stain:
   100 mM sodium phosphate, pH 7.3
   (77 mM Na_2HPO_4, 23 mM NaH_2PO_4)
   1.3 mM $MgCl_2$
   3 mM potassium ferricyanide ($K_3Fe[CN]_6$)
   3 mM potassium ferrocyanide ($K_4Fe[CN]_6$)
   1 mg/mL X-Gal
2. Filter through 0.45 μm membrane prior to use.
3. Overlay fixed cells with X-Gal stain.
4. Place in a humidified incubator at 37 °C.
5. Monitor for blue color development (from 30 minutes to overnight).

As a matter of preference, the concentrations of potassium ferricyanide and potassium ferrocyanide may be as high as 35 mM. At higher concentrations, the indole precipitation occurs more quickly and helps to reduce diffusion. However, these concentrations may cause a greenish background upon prolonged incubation in some tissues.[3] One publication suggests that using X-Gal solutions at pH >7.5 can help to eliminate endogenous mammalian β-galactosidase activity.[15]

Related Products

Catalog Number GALS: β–Galactosidase reporter gene staining kit, X-Gal based

Catalog Number N1127: *o*-Nitrophenyl β-D-galactopyranoside (ONPG), soluble substrate for β-galactosidase

Catalog Number GALA: β-Galactosidase reporter gene activity detection kit, ONPG-based

Catalog Number M1633: 4-Methylumbelliferyl β-D-galactopyranoside (MUGal), fluorescent substrate for β-galactosidase

Catalog Number B3928: Blue-White Select screening reagent, ready-to-use IPTG and X-Gal solution in DMSO

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