Beijing Solarbio Science & Technology Co., Ltd
Tel: 010-56371207
Fax: 010-56371281/82
Http://www.solarbio.cn

X-gal

CAS Number: 7240-90-6

Storage Temperature: -20°C

Product Description :

Appearance: White powder

Molecular formula: C14H15BrClNO6

Molecular Weight: 408.64

Synonyms: 5-Bromo-4-Chloro-3-Indolyl-beta-D-Galactopyranoside

5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside, commonly known as X-Gal, is a histochemical substrate for β-galactosidase. X-Gal is cleaved by β-galactosidase to yield an insoluble blue precipitate. X-Gal is particularly useful in molecular biology applications to detect the activity of β-galactosidase which is frequently used as a reporter gene. 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. Insertion of DNA into the lacZ region results in the loss of β-galactosidase activity. Lac+ bacterial colonies resulting from a-complementation will appear blue whereas bacterial colonies containing plasmid with DNA inserted in the lacZ region, will be incapable of α-complementation and will appear white.

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

Preparation Instructions:

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

Storage/Stability:

Stored at -20°C protected from light X-Gal powder can have a three year shelf life.

Precautions and Disclaimer:

For Laboratory Use Only. Not for drug, household or other uses.

Procedures:

Blue/White Colony Screening

a)X-Gal included in agar: Add 5 ml of X-Gal stock solution and 5 ml of 0.1 M isopropyl-β-D-thiogalactoside (IPTG) for each 1 liter of autoclaved media agar (e.g. LB agar,) containing appropriate antibiotics just

prior to pouring. The media should be below 55°C. Plate cells on cooled agar and incubate overnight at 37°C.

b) X-Gal applied to top of agar: To a premade LB agar plate (e.g. prepared using LB agar), add 40 µl of X-Gal stock solution (at room temperature) and 4 µl of a 200 mg/ml solution of IPTG. Spread solution over the entire surface of the plate. Incubate at 37°C until the the fluid is no longer visible. This may take several hours. 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.

Immunocytochemistry

Prepare X-Gal Stain: 100 mM sodium phosphate, pH 7.3 (77 mM Na2HPO4, 23 mM NaH2PO4), 1.3 mM MgCl2, 3 mM potassium ferricyanide (K3Fe[CN]6), 3 mM potassium ferrocyanide (K4Fe[CN]6), 1 mg/ml X-Gal. Filter through 0.45 µm membrane prior to use. Overlay fixed cells with X-Gal stain. Place in a humidified incubator at 37°C and 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. A recent publication suggests that using X-Gal solutions at pH greater than 7.5 can help to eliminate endogenous mammalian β-galactosidase activity.