

Description

5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-GAL) is a chromogenic substrate for β -Galactosidase that forms an intense blue precipitate. It can be used in molecular biology to detect the *gal* gene product, and also in microbiology where it is used to detect micro-organisms which have β -Galactosidase activity (usually coliforms). It can be combined with the R-substrates to differentiate between two species of organisms on the same plate. X-GAL is soluble in N, N-dimethylformamide or dimethylsulfoxide (DMSO). Together with appropriate bacterial cloning vectors, host strains, and IPTG, X-GAL provides an easy way to distinguish between positive and negative clones after transformation.

| Features | Applications | | |
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| • Extremely pure, 99.5% by TLC (Thin Layer Chromatography) | Blue/White colony screening to distinguish recombinant (white) and non-recombinant (blue) colonies | | |
| Intense blue precipitate upon hydrolysis | Immunoblotting | | |
| | Detect β-galactosidase activity in immunological and histochemical assays | | |
| | Microbiology and cell culture media | | |
| Protocol | Product Citations | | |
| 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 | Wilson, A.C., <i>et al. J. Bacteriol.</i> 190(15), 5522-5525(2008). Liapis, E., <i>et al. NAR</i> 36(18), 5933-5945 (2008). Corbett, D., <i>et al. J. Biol. Chem.</i> 282, 33326-33335 (2007). Toledo-Arana, A., <i>et al. J. Bacteriol.</i> 187(15), 5318-5329 (2005). | | |

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-b-D-thiogalactoside (IPTG, BIO-37082) for each 1L of autoclaved media agar (e.g. LB agar) containing appropriate antibiotics just before 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 μ l of X-Gal stock solution (at room temperature) and 4 μ l of a 200 mg/ml solution of IPTG.

Spread solution over the entire surface of the plate. Incubate at 37 $^{\circ}$ C until the fluid is no longer visible. This may take several hours. Plate cells and incubate overnight at 37 $^{\circ}$ C.

Associated Products

| IPTG Powder | BIO-37036 | |
|--------------------|-----------|--|
| Quick-Stick Ligase | BIO-27027 | |
| SureClean Plus | BIO-37047 | |

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