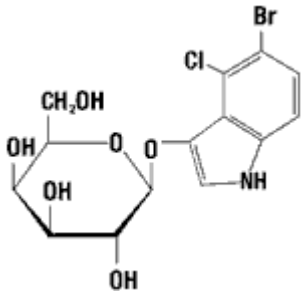
X-Gal

（5–Bromo–4–Chloro–3–Indolyl–β–D–Galactoside）

Formula: $C_{14}H_{15}Br\ Cl\ N\ O_6$
Molecular Weight:408.6



Store: Store at -20°C in the dark.
Code No.:ZB136
Package:50g
Price:EUR(€)1000.00

Description

X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside) is an inert chromogenic substrate for beta-galactosidase, an enzyme that promotes lactose utilization. Beta-galactosidase hydrolyzes X-Gal into a colorless galactose and 4-chloro-3-brom-indigo which forms an intense blue precipitate. Induction of the *lacZ* gene with IPTG leads to the hydrolysis of X-Gal and to the development of blue colonies (*see* the scheme below).

Applications：

- Blue/white colony screening assay, distinguishing recombinant colonies (white) among non-recombinant ones (blue), *see* Protocol for the Blue/White Colony Screening.
- Visualization of expression of the beta-galactosidase reporter gene in transfected eukaryotic cells, *see* Protocol for the Detection of the Beta-galactosidase Reporter Gene in Transfected Eukaryotic Cells.
- Detection of beta-galactosidase activity in immunological and histochemical procedures.

Add: Floor 2 ,Building A,328# Wuhe Road, Shanghai 200233 China
Tel: +86-21-54460832 Web: www.synthesisgene.com
Fax:+86-21-54460831 E-mail: master@shinegene.org.cn

Quality Control：
Greater than 98% purity confirmed by HPLC.
Functionally tested in blue/white colony screening.

Note

- Preparation of a 20 mg/ml stock solution in dimethylformamide or dimethylsulfoxide is recommended.
- Dimethylformamide dissolves some plastic materials. The direct addition of dimethylformamide containing solution to plastic Petri dishes should be avoided.

Protocol for the Blue/White Colony Screening

For individual LB (Luria Broth) agar plates:

1. Pour sterile warm LB agar (about 25 ml) into a Petri dish.
2. Dry opened LB plates at room temperature under UV light for about 30 min.
3. Add 40 µl of the X-Gal Solution (20 mg/ml), ready-to-use.
4. Add 40 µl of 100 mM IPTG Solution, ready-to-use.
5. Spread evenly on the plate with a sterile spatula.

For batch use, add the following directly per 1 ml of the liquid LB agar (kept at about 50°C):

1. 1 µl of X-Gal Solution (20 mg/ml), ready-to-use.
2. 1 µl of 100 mM IPTG Solution, ready-to-use.
3. Mix well.
4. Pour 25 ml of prepared LB agar into each Petri dish.
5. Dry opened LB plates at room temperature under UV light for about 30 min.

Protocol for the Detection of the Beta-galactosidase Reporter Gene in Transfected Eukaryotic Cells

Buffers:
10X PBS buffer (pH 7.4): 1.37 M NaCl, 0.27 M KCl, 1 M Na_2HPO_4, 0.02 M K_2HPO_4.
Fixation buffer (pH 7.4): 1X PBS buffer and 0.25% glutardialdehyde.
Staining buffer, prepare immediately before use as follows:

Stock solutions	Volume per 10 ml staining buffer	Final concentration
1 M $MgCl_2$	20 µl	2 mM
0.5 M $K_4Fe(CN)_63H_2O$	100 µl	5 mM

0.5 M $K_3Fe(CN)_6$	100 µl	5 mM
X-Gal (20 mg/ml) in dimethylformamide	500 µl	1 mg/ml
10X PBS buffer (pH 7.4)	9.28 ml	diluted 10-fold

Staining procedure:

1. Wash the cells twice with cold 1X PBS buffer. Adhered cells can be washed in the transfection plates, suspension cells should be pelleted before washing.
2. Fix the cells with Fixation buffer for 10 minutes at room temperature while gently rocking the plate. Use 150 µl of the Fixation buffer for each well of a 24-well plate.
3. Wash the cells twice with cold 1X PBS buffer.
4. Stain the cells with freshly prepared Staining buffer for 2-20 hours at 37°C. Use 200 µl of Staining buffer for each well of a 24-well plate.
5. Count dark blue cells.

Shanghai ShineGene Molecular Bio-tech Co.,Ltd.

Add：Floor 2 ,Building A,328# Wuhe Road, Shanghai 200233

Tel：+86-21-54460832

Fax:+86-21-54460831

E-mail:master@shinegene.org.cn

Website：www.synthesisgene.com