

## Product Information

# 5-Bromo-4-chloro-3-indolyl $\beta$ -D-galactopyranoside

Tablet

**B6024**

## Product Description

Synonyms: BCIG, X-Gal, 5-Bromo-4-chloro-3-indolyl  $\beta$ -D-galactoside (5-Bromo-4-chloro-3-indolyl  $\beta$ -D-galactopyranoside component)

CAS Registry Number: 7240-90-6 (X-Gal component)

Molecular Formula:  $C_{14}H_{15}BrClNO_6$  (X-Gal component)

Molecular Weight: 408.63 (X-Gal component)

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

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

Many other applications also use X-Gal as a substrate to detect  $\beta$ -galactosidase activity, such as:

- $\beta$ -galactosidase-antibody-linked immunoassays and immunohistochemistry<sup>6-9</sup>
- coliphage detection based on  $\beta$ -galactosidase induction<sup>10</sup>
- the detection of micrometastasis formation during tumor progression<sup>4</sup>

Several dissertations cite use of B6024 in their protocols.<sup>11-15</sup>

## Reagent

This product is in tablet form, with 5 mg X-Gal substrate per tablet.

X-gal is available in several other forms:

- Cat. Nos. B4252 and B9146 are in powder form.
- The Molecular Biology Reagent products, Cat. Nos. B9146 and B6024, are quality control-tested for identification of lac<sup>+</sup> bacterial colonies.

(See also the Related Products section.)

## Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

Store the tablets at  $-20$  °C. When stored at  $-20$  °C, the tablets are stable for at least one year. Tablets are good as long as their color remains white.

## Preparation Instructions

Prepare a stock solution of X-Gal, at the equivalent of 20 mg/mL, in either *N,N*-dimethylformamide (DMF) or dimethylsulfoxide (DMSO).<sup>16,17</sup> Sterilization is not required.<sup>3</sup> Store stock solutions in glass containers protected from light at  $-20$  °C.<sup>18</sup> Solutions may be stored at  $-20$  °C for 6-12 months. If a solution turns pink, it should be discarded.

## Procedure

### Blue/White Colony Screening

#### X-Gal included in agar

1. Add 5 mL of X-Gal stock solution and 5 mL of 0.1 M isopropyl- $\beta$ -D-thiogalactoside (IPTG, such as Cat. No. I6758) for each 1 L of autoclaved medium agar (such as LB agar, such as Cat. No. L2897) containing appropriate antibiotics just prior to pouring.<sup>19</sup> The temperature of the medium should be < 55 °C.
2. Plate cells on cooled agar.
3. Incubate overnight at 37 °C.

#### X-Gal applied to top of agar

1. To a premade LB agar plate (such as prepared using LB agar, such as Cat. No. L2897), add 40  $\mu$ L of X-Gal stock solution (at room temperature) and 4  $\mu$ L of a 200 mg/mL solution of IPTG.<sup>5</sup>
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.<sup>5</sup>

### Immunocytochemistry<sup>2,3,4,9</sup>

1. Prepare X-Gal Stain:
  - 100 mM sodium phosphate, pH 7.3 (77 mM Na<sub>2</sub>HPO<sub>4</sub>, 23 mM NaH<sub>2</sub>PO<sub>4</sub>)
  - 1.3 mM MgCl<sub>2</sub>
  - 3 mM potassium ferricyanide (K<sub>3</sub>Fe[CN]<sub>6</sub>)
  - 3 mM potassium ferrocyanide (K<sub>4</sub>Fe[CN]<sub>6</sub>)
  - 1 mg/mL X-Gal
2. Filter through a 0.45  $\mu$ m 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.<sup>3</sup> One publication suggests that using X-Gal solutions at pH >7.5 can help to eliminate endogenous mammalian  $\beta$ -galactosidase activity.<sup>20</sup>

## Related Products

Cat. No. GALS:  $\beta$ -Galactosidase reporter gene staining kit, X-Gal based

Cat. No. N1127: *o*-Nitrophenyl  $\beta$ -D-galactopyranoside (ONPG), soluble substrate for  $\beta$ -galactosidase

Cat. No. M1633: 4-Methylumbelliferyl  $\beta$ -D-galactopyranoside (MUGal), fluorescent substrate for  $\beta$ -galactosidase

Cat. No. B3928: Blue-White Select screening reagent, ready-to-use IPTG and X-Gal solution in DMSO

## References

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