Invitrogen™
life technologies

Product: Bluo-Gal (M.W. 373.19)

Cat. No.: 15519-028 **Storage Conditions:** -20°C
Lot No.: _____
Size: 1 gram

BACKGROUND:

Bacterial Systems
Bluo-Gal is an alternative histochemical substrate for ß-galactosidase that produces a darker blue color than the traditional substrate, X-gal. As with X-gal, Bluo-Gal produces blue (lac^+) bacterial colonies and blue (lac^+) viral plaques.

Immunodetection Systems
Bluo-Gal can also be used as a substrate with the Streptavidin-ß-galactosidase conjugate in both immunoblot and immunocytochemistry assays. In the presence of ß-galactosidase, Bluo-Gal yields a blue precipitate, which is insoluble in alcohols and xylenes.

NOTES: Bluo-Gal may be used like X-Gal (Cat. No. 15520) for detection of blue (lac^+) bacterial colonies or blue (lac^+) virus plaques. To achieve optimal performance of Bluo-Gal the following conditions are necessary when using Bluo-Gal on bacterial plates.

1) Media must be buffered to pH 7.0 - 8.0.
2) Bluo-Gal must be used at a final concentration of at least 300 µg/ml in top agar and autoclaved media.
3) Bluo-Gal should be added to autoclaved media after it has cooled to 45-55°C.
If these three parameters are not adjusted properly then minimal color will be produced by Bluo-Gal.

INSTRUCTIONS FOR USE:

For Detection of Lac^+ Colonies (E. coli) or M13 mp viral plaques:

Bluo-Gal is prepared as a 2% (20 mg/ml) solution in dimethylformamide. This solution may be stored in a glass tube with a screw cap wrapped in foil at 4°C for several weeks or -20°C for 6 months.

The following amounts of Bluo-Gal are recommended for use:

1. With top agar overlay protocol for M13 cloning use 50 µl of the 2% Bluo-Gal solution per 2.5 ml of top agar.
2. For spreading on the surface of plates, use 50-100 µl of the 2% Bluo-Gal stock solution.
3. For autoclaved media, add 420 µl of the 2% Bluo-Gal stock per 25 ml of media.

(Instructions Continued on Reverse Side)

QUALITY CONTROL DATA: See back page.

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QUALITY CONTROL DATA:

Each lot of Bluo-Gal is assayed for chemical purity and function by two methods:
HPLC analysis (% purity): > 98%
Test as substrate for ß-galactosidase in solution phase: royal blue

PROTOCOL FOR USING BLUO-GAL IN TOP AGAR OVERLAY FOR M13mp TRANSFECTION ASSAY

Media for top agar:	Bacto-tryptone	10 g/liter
	NaCl	8 g/liter
	Adjust pH to 7.5 with HCl or NaOH	
Soft (top) Agar:	Add 6 gm of agar to 1 liter of the above media and autoclave.	
Agar Plates:	Bacto-agar	20 g/liter

Autoclave and pour into petri dishes (25 ml/plate). Prior to pouring the dish check the final pH of the combined agar and medium with pH paper and adjust to 7.5 with HCl or NaOH. Dry and store the agar plates at 4°C.

1. Add the appropriate amount of DNA (1-10 ng) to 100 µl of competent M13 host cells in a 13x100 glass tube and let the mixture sit on ice for 30 minutes. Heat shock the mixture for 2 minutes at 42°C and place on ice.
2. To this DNA/cell mixture, add 150 µl of exponentially growing M13 host cells. Mix and incubate at 37°C for 5 minutes.
3. To 2.5 ml of top agar at 42°C, add the following components:

   10 µl 100 mM IPTG (Invitrogen, 15529-019)
   50 µl 2% Bluo-Gal in dimethylformamide
4. Now add the mixture of cells and DNA from Step 2 to the top agar from Step 3. Mix and immediately place on prepared agar plates.
5. Let the agar solidify, then invert plates, and incubate at 37°C until plaques are seen (12-18 hrs.).

IMMUNODETECTION PROTOCOLS

Bluo-Gal can be used effectively with a ferric cyanide system. This system described as the "iron" system generally works quickly and gives an intense color. The ferric cyanide present in the substrate solution rapidly oxidizes the enzyme-generated indole derivative to give the blue indole dimer.

"IRON" (FERRIC) CYANIDE SYSTEM

Solution A: Dissolve Bluo-Gal in dimethylformamide to a final concentration of 20 mg/ml (2% solution)
Solution B: 3.1 mM potassium ferricyanide
3.1 mM potassium ferrocyanide
10 mM sodium phosphate, pH 7.2
0.15 M NaCl
1.0 mM $MgCl_2$
(Both solutions A and B can be stored at -20°C for at least six months.)

Working Solution:	0.05 ml	Solution A
	2.3 ml	Solution B

The working solution can be stored at -20°C for up to 1 month.

USE: Immerse slides or filters in the substrate working solution. Incubate for 1 hour at 28-37°C. Wash thoroughly with Phosphate Buffered Saline PBS (slides) or distilled water (blots). Counterstain slides with neutral red if desired. Slides may be permanently mounted for viewing.

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