MarkerGene™ β-Galactosidase Staining Kit
(Product M1352)
Product Information Sheet (M1352-006)
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NOTE: The following information is given as a viable methodology for use of the MarkerGene™ β-Galactosidase Staining Kit. The user may determine their own best conditions for use dependent upon the specific conditions present in their experiment.

I. Overview
The E. coli lacZ gene is among the most widely used marker genes for mammalian, yeast and bacterial transfection studies due in part to its resistance to intracellular proteolytic degradation. The lacZ gene encodes for the β-Galactosidase (β-Gal) enzyme that catalyzes the hydrolysis of a wide range of β-galactosides. The MarkerGene™ β-Galactosidase Staining Kit can be used to monitor and detect this enzyme activity in stably or transiently transfected cells or transgenic tissues using the sensitive chromogenic substrate 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). When β-Gal cleaves the glycosidic linkage in X-Gal, a soluble, colorless indoxyl derivative is produced, which quickly dimerizes and becomes oxidized to produce a bright blue indigo dye precipitate at the site of β-Gal activity. The dimerization and oxidation reaction is facilitated, in this kit, by a developer buffer solution containing ferric and ferrous ions. Both transfection efficiency and comparative expression levels can be determined by examining and counting the number of blue cells in the total cell population. The kit provides enough reagents and standards for up to 100 assays, using a microtiterplate method, and includes a detailed protocol for use in cultured mammalian cells, as well as yeast and bacterial samples.

II. Materials
A. Substrate: 50 mg 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) in 2.5 ml Dimethylformamide (DMF).

B. Staining Buffer (25 ml): This solution contains 100 mM MgCl₂, 1 M sodium phosphate, pH 7.0, ferric and ferrous ions.

C. Fixing Solution (80 ml): This solution contains 100 mM MgCl₂, 25% glutaraldehyde, and 1 M sodium phosphate, pH 7.0.

D. 10X Phosphate Buffered Saline (PBS) (30 ml): Dilute 1:10 with distilled DI water to provide 1X PBS

E. Storage and Handling: Store X-Gal at -20°C. Fixing solution should be handled with care and proper precautions taken as it contains 25% glutaraldehyde; see MSDS for more information; store solution at 4°C. Store Staining Buffer at room temperature.

F. Additional Items Required:
III. Protocol
For 6 well tissue culture plate (35 mm)

1) Healthy exponentially growing cells are cultured to 70% confluency using recommended cell culture conditions for your particular cell line. Cells may be grown on sterile (18mm) cover slips and transferred to six-well plates for staining, if desired.

2) Aspirate culture medium from cells in each well.

3) Add 0.8 ml cold Fixing Solution (M1352-003) (0.01% Glutaraldehyde in 1 M Sodium Phosphate, pH 7.0) to each well, enough to cover cells. Incubate for 10 minutes at 37°C.

4) Prepare X-Gal Staining Solution. Dilute thawed X-Gal solution 1:9 in Staining Buffer. **Note:** Only a small amount (250 µl) of Staining Solution with X-Gal is needed per 35 mm well. Make only as much as needed since the Staining Solution with X-Gal loses activity when stored.

5) Remove Fixing Solution from cells and rinse 3 times with 1 ml 1X PBS. Thorough rinsing is needed to remove Fixing Solution to prevent inhibition of enzyme reaction.

6) Add 250 µl Staining Buffer with X-Gal to each 35 mm well, enough to cover cells. Incubate at 37°C for 30-120 minutes or longer if necessary. Longer incubation times provide darker staining patterns. Cells may be stained for up to 18 hours, if transfection levels are low, **Note:** Prolonged staining can result in false-negatives. Adjust staining time to provide the shortest time possible for reliable results.

7) Examine cells using a light microscope. In randomly selected fields of view, count the number of stained verses unstained cells. Photograph cells, if possible, for later evaluation.

8) Calculate the percentage of β-Gal expressing cells (stained cells) in the total population using the formula below:

   \[
   \text{Number of Blue Cells} \times 100\% = \text{Percent of cells containing } \beta\text{-galactosidase} \\
   \text{Total number of Cells}
   \]
Figure 1: Different ratios 100:0, 80:20, 50:50, 20:80, 0:100 of CREBAG2:NIH3T3 murine endothelial cells were cultured in Dulbecco’s Modified Eagle’s (DME) Medium containing 9% fetal calf serum (FCS) and 1X antibiotic/antimycotic (Gibco, 15240-062). The cells were fixed and stained according to the protocol. After adding the Staining Buffer the cells were incubated at 37°C for two hours. Using a light microscope, the total number of cells and stained (blue) cells were counted. The percent of cells containing β-galactosidase were calculated according to the formula in step 8 of the protocol and related to the initial ratio (percentage) of CREBAG2 cells in the original sample.

Figure 2: (a) 100:0 CREBAG2:NIH3T3; (b) 80:20 CREBAG2:NIH3T3; (c) 50:50 CREBAG2:NIH3T3; (d) 20:80 CREBAG2:NIH3T3; (e) 0:100 CREBAG2:NIH3T3. All cells were fixed and stained according to above protocol.
## M1352: Kit Contents

<table>
<thead>
<tr>
<th>Description</th>
<th>Quantity</th>
<th>Part No.</th>
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<tbody>
<tr>
<td><strong>Reagents</strong></td>
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<tr>
<td>5-BROMO-4-CHLORO-3-INDOLYL-B-D-GALACTOPYRANOSIDE (X-GAL)</td>
<td>50 mg / 2.5 mL DMF</td>
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<td>Staining Buffer</td>
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<td>RT</td>
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<td>Fixing Solution</td>
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<td>C, R</td>
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<td>10X Phosphate Buffered Saline (PBS)</td>
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Notes: F=store at or below -20°C; C=store cold (4°C); RT=store at room temperature; R=read protocol instructions carefully prior to use.
REFERENCES