1. Introduction

The lacZ gene of *E. coli*, encoding for β-galactosidase (β-Gal), is being used extensively as a reporter of gene expression in a variety of systems, including bacteria and yeast (1), mammalian (2–6), avian (7), and insect (6,8) cells, as well as in whole insects (9) and nematodes (10). The enzymatic activity of bacterial β-Gal can be assayed readily from transfected cells and tissue. It exhibits maximal enzymatic activity at neutral to alkaline pH (optimal pH 7.0 – 7.5), which makes discrimination from endogeneous mammalian β-galactosidase (optimal pH 3.0 – 6.0) possible (11). β-Gal has become a preferred reporter for normalization in co-transfection experiments, especially due to the colorimetric substrates ONPG and CPRG, and their ease of use for determining of β-Gal activity in cell extracts. More recently, the availability of methods for highly sensitive detection of β-galactosidase by chemiluminescent reporter gene assays* and a standardized β-Gal ELISA* have led to an even more wide-spread use of β-Gal reporter constructs.

Another advantage of the β-Gal system is the availability of the precipitating β-Gal substrate 3-indolyl-β-D-galactopyranoside (X-Gal), classically used in prokaryotic clone selection procedures. In combination with a specific iron buffer, X-Gal offers an easy-to-use histochemical procedure, enabling detection of individual cells expressing a transfected bacterial lacZ gene by light microscopy. It thereby yields a blue stain within positively transfected cells without producing any background. Histochemical staining is useful for determining the percentage of transfected cells, and specifically to optimize transfection conditions. It may also be used for staining of tissue sections, such as evaluating in vivo transfection efficiencies in animal model systems (12, 13).

Histochemical β-Gal staining is also useful to detect β-galactosidase conjugated to antibodies in immunohistochemical procedures (14, 15). The β-Gal Staining Set developed by Roche Molecular Biochemicals combines specially stabilized formulations of X-Gal and an iron buffer, that are ready to use upon mixing.

The β-Gal Staining Set offers the following advantages

- **Time-saving** (staining solution prepared by the simple mixing of two solutions)
- **Convenient** (stabilized solutions allow storage at +2 to +8°C)
- **Safe** (avoids the hazards associated with dimethylformamide, commonly used for preparing X-Gal solutions)

2. Product description

**Set components**

<table>
<thead>
<tr>
<th>Vial</th>
<th>Content</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>X-Gal solution, stock solution in dimethyl sulfoxide (DMSO). <strong>Note:</strong> DMSO-containing solutions may solidify upon storage at +2 to +8°C. If this occurs, incubate vial at +37°C in a waterbath and vortex until solution appears clear.</td>
<td>6 ml</td>
</tr>
<tr>
<td>2</td>
<td>Iron buffer, potassium ferrocyanide and potassium ferricyanide in phosphate buffered saline (PBS), stabilized</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

**Stability**
The product is stable at +2 to +8°C until the control date indicated (see lot-specific data on the label imprint).

**Specificity**
The β-Gal Staining Set allows specific detection of bacterial lacZ gene encoded β-galactosidase.

3. Application

Upon transfecting cells with a β-Gal encoded construct, (e.g., using DOTAP Liposomal Transfection Reagent*), cells are fixed, washed in PBS and stained with freshly prepared staining solution. After staining, cells are washed again in PBS and evaluated by microscopy. When long-term storage is desired, PBS is replaced by mounting media (e.g., glycerol).

For immunohistochemical staining of cells or tissue sections, incubate the specimen with an antibody conjugated to bacterial β-galactosidase, according to standard protocols. Subsequently, the specimen is processed for detection of β-Gal as described for transfected cells.

4. Preparation of the staining solution

- **Examine the X-Gal solution, as it should be clear** before use. DMSO-containing solutions may solidify upon storage at +2 to +8°C. If this occurs, incubate vial 1 at +37°C in a waterbath and vortex until solution appears clear.
- **Dilute 1 part of X-Gal solution with 19 parts of Iron Buffer** before use. Do not store.

**Table 1:** Required volumes of staining solution per well or dish

<table>
<thead>
<tr>
<th></th>
<th>24-well plate</th>
<th>6-well plate</th>
<th>6 cm dish</th>
<th>10 cm dish</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-Gal Solution</td>
<td>25 μl</td>
<td>50 μl</td>
<td>100 μl</td>
<td>150 μl</td>
</tr>
<tr>
<td>Iron Buffer</td>
<td>475 μl</td>
<td>950 μl</td>
<td>1900 μl</td>
<td>2850 μl</td>
</tr>
<tr>
<td>Total volume</td>
<td>0.5 ml</td>
<td>1.0 ml</td>
<td>2 ml</td>
<td>3 ml</td>
</tr>
</tbody>
</table>

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5. Procedure

Additionally solutions required

<table>
<thead>
<tr>
<th>Fixative</th>
<th>24-well plate</th>
<th>6-well plate</th>
<th>6 cm dish</th>
<th>10 cm dish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixative</td>
<td>0.5 ml per well</td>
<td>1 – 2 ml per well</td>
<td>2 – 4 ml per dish</td>
<td>3 – 5 ml per dish</td>
</tr>
</tbody>
</table>

- **PBS** (phosphate buffered saline)
- **Mounting medium** (optional): e.g., 70% glycerol in PBS.

**Staining transfected cells** (example for 3.5 cm culture dishes (6-well plate))

- Transfect cells (e.g., using DOTAP Liposomal Transfection Reagent*) with a β-galactosidase encoding construct, as described in the respective pack insert.
- Wash cells once with PBS.
- Remove PBS and add 1 – 2 ml fixative per well of a 6-well culture plate. Incubate for 15 min at + 15 to + 25°C.
- Remove fixative and wash cells 3 times with PBS.
- **Note:** If required, the fixed and washed cells may be stored at +2 to +8°C in PBS, for up to 1 week before staining.
- Add 1 ml staining solution per well of a 6-well culture plate, and incubate for 0.5 – 3 h at +37°C until cells are stained blue. Check for color development under microscope and continue incubation if necessary.
- **Note:** Especially, when analyzing highly confluent cells, monitor incubation time closely. Staining for too long may result in false-positive results, with unspecific binding of antibodies.

6. References

- 16 Contact and Support for life science research only. Not for use in diagnostic procedures.

Contact and Support

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