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I. Introduction

The **Beta-Galactosidase Staining Kit** (Cat. No. 631780) provides a complete set of reagents for X-gal detection of beta-galactosidase expression in mammalian cells.

II. Required Materials

Supplied in Kit:

- X-Gal Solution (20 mg/ml)
- Staining Solution 1*
- Staining Solution 2*
- Staining Solution 3
- 10X Fixing Buffer*
- 10X PBS

*__CAUTION:__ Due to their toxic properties, wear gloves and take appropriate precautions (see MSDS) when using Fixing Buffer (Section III, Steps 2–5) and Staining Solutions 1 and 2 (Section III, Steps 4–8).

Not Supplied:

- Deionized H_2O
- 37°C incubator
- Light microscope
- 35 mm cell culture dishes

III. Staining Protocol (35 mm Dish Format)

1. Gently wash the cells with 2 ml of 1X PBS in a 35 mm* cell culture dish.

   * Scale up as needed if you are using a larger dish.

   NOTE: Washing too vigorously can cause detachment of less adherent cells.

2. Add 2 ml of 1X Fixing Buffer* to the dish.

   * Prepare 1X Fixing Buffer by diluting 200 µl of 10X Fixing Buffer 1:10 with deionized H_2O.

3. Incubate 5–10 minutes at room temperature.

   NOTE: Longer fixation times can result in enzyme inactivation and loss of signal.

4. While the cells are fixing, prepare the appropriate amount of X-Gal Staining Mix (Table I).

   Table 1. Preparing X-Gal Staining Mix

Component	1 Dish	6 Dishes
X-Gal Solution	100 µl	600 µl
Staining Solution 1	20 µl	120 µl
Staining Solution 2	20 µl	120 µl
Staining Solution 3	20 µl	120 µl
1X PBS	1.84 ml	11.04 ml
Total Volume	2 ml	12 ml

   NOTE: If there is a visible precipitate after thawing the staining solutions, warm to 37°C and vortex until dissolved.

5. Wash the dish twice with 2 ml of 1X PBS.

   NOTE: Thorough washing is necessary to remove the Fixing Buffer in order to prevent inhibition of the enzyme reaction.

6. Add 2 ml of the X-Gal Staining Mix to the dish.
7. Incubate the dish at 37°C for 1 hr to overnight.
8. Assay for development of blue color using light microscopy.

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