Protocol-at-a-Glance

Luminescent β-galactosidase Reporter System 3 Protocol-at-a-Glance

(PT2106-2)

Please read the *User Manual* before using this abbreviated protocol. The Protocol-at-a-Glance is provided for your convenience, but is not intended for first-time users.

A. Preparation of cell lysates for assay of β-galactosidase

Maximal levels of β-galactosidase are generally detected 48–72 hr after transfection. The volumes listed below are for adherent cells cultured on 60-mm tissue culture plates. If working with suspended cell cultures, simply collect an equivalent cell mass by centrifugation, wash three times by resuspending in 500 µl of ice-cold PBS followed by centrifugation, and proceed from Step 6.

1. Wash cells on the plate twice with 4.0 ml of ice-cold PBS.
2. Add 1.0 ml of ice-cold PBS to each plate.
3. Scrape cells off the plate using a rubber policeman (or equivalent), and transfer cell suspension to a microcentrifuge tube on ice.
4. If residual cells are still on the plate, use another 500 µl of PBS to collect the remaining cells and transfer to the same microcentrifuge tube.
5. Centrifuge at maximum speed for 15 sec in a microcentrifuge.
6. Aspirate supernatant, being careful not to disturb the cell pellet.
7. Gently resuspend cells in 1.0 ml of ice-cold PBS.
8. Repeat Steps 5 and 6, and carefully aspirate the last traces of PBS.
   Note: The cell pellet can be stored at –20°C for 1–3 days with minimal loss of β-galactosidase activity.
9. Gently resuspend the cell pellet in 75 µl of ice-cold lysate buffer.
10. Place the tube with cell suspension in a dry-ice/ethanol bath for 1 min (or until completely frozen).
11. Thaw the cell suspension at 37°C for 1–2 min.
12. Repeat the freeze/thaw cycle (Steps 10–11) two more times.
13. Centrifuge at 4°C for 5 min at maximum speed.
14. Transfer the supernatant to a fresh tube and keep on ice.

B. Chemiluminescent β-galactosidase assay using a tube luminometer*

1. Warm enough Reaction Buffer and Reaction Substrate to room temperature.
2. Prepare the master Reaction Buffer Mixture by adding 4 µl of Reaction Substrate to 196 µl of Reaction Buffer for each sample.
3. Aliquot 30–50 µl of individual cell lysates into sample tubes.
4. Add 200 µl of Reaction Buffer Mixture to each cell lysate and mix gently.
5. Incubate at room temperature (20–25°C) for 60 min.
6. Record light emission as a 5-sec integral using a tube luminometer.
   Note: Since the light signal is stable for >1 hr, the emission can be recorded 0–60 min after the incubation.

*Consult the main protocol for information on detection using a plate luminometer or x-ray film.

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