Glycerol-Glo™ Assay

Instructions for Use of Products J3150 and J3151.

Quick Protocol

This document is a quick protocol for the calculation of glycerol levels in biological samples. For complete protocol information, including information on standard curve preparation, see the *Glycerol-Glo™ Assay Technical Manual* #TM599, which is available online at: **www.promega.com/protocols/**

The protocol is for a reaction with 50µl of a prepared sample and 50µl of Glycerol Detection Reagent in a 96-well plate. The assay can be adapted to other volumes provided the 1:1 ratio of Glycerol Detection Reagent volume to prepared sample volume is maintained.

Protocol

1. Reagent Preparation:
    a. Thaw all components in a 22°C water bath and mix to ensure homogeneous solutions prior to use. Place the Reductase Substrate and Kinetic Enhancer on ice; all other components can be held at 22°C until use.
    b. Determine the amount of reagents necessary for the current experiment. Use reagents on the day they are prepared; do not store prepared reagents for later use.
    c. To prepare Glycerol Detection Reagent, add 10µl of Reductase Substrate per ml of Glycerol Detection Solution and mix by inversion. Prepare this reagent 1 hour before use in order to minimize assay background. Hold at room temperature.
    d. After 1 hour, add 10µl of Kinetic Enhancer per ml of Glycerol Detection Reagent prepared in Step c and mix by inversion.
2. Prepare samples, standards and controls:
    a. For Medium, Serum and Homogenized Tissue Samples:
        i. Dilute samples in Glycerol Lysis Solution to bring their glycerol concentrations below 80µM.
        ii. Transfer 25µl of sample, standard or control to a 96-well plate.
        iii. Add 25µl of Glycerol Lysis Solution, shake briefly and incubate 30 minutes at 37°C.
    b. For Adherent Cells and 3D Cell Cultures:
        i. Remove medium from cells in the 96-well plate. Wash cells twice with 100µl of PBS.
        ii. Add 50µl of Glycerol Lysis Solution, shake briefly and incubate 30 minutes at 37°C.
        iii. Dilute samples in Glycerol Lysis Solution to bring their glycerol concentrations below 80µM.
        iv. If needed, dilute samples in Glycerol Lysis Solution to bring their glycerol concentrations below 80µM. Add 50µl of any diluted samples, standards or controls to empty wells in a 96-well assay plate.
3. Add 50µl of Glycerol Detection Reagent to all wells.
4. Shake the plate for 30–60 seconds by hand or using a plate shaker at a low rpm.
5. Incubate the plate at room temperature for 1 hour.
6. Record luminescence on a plate-reading luminometer.
7. Calculate glycerol concentrations by comparison of the luminescence of samples and standards prepared under the same conditions.

Additional protocol information is in Technical Manual #TM599, available online at: **www.promega.com**

