Glycerol-Glo™ Assay

Instructions for Use of Products J3150 and J3151.

Quick Protocol

This quick protocol is for 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, 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.

Glycerol is a common laboratory reagent. Be careful to avoid glycerol contamination of assay components.

Preparing Reagents

- Thaw all components in a 22°C water bath. Mix prior to use. Place the Reductase Substrate and Kinetic Enhancer on ice; all other components can be held at 22°C until use. Use reagents on the day they are prepared, only.
- **Glycerol Detection Reagent:** Add 10µl of Reductase Substrate per ml of Glycerol Detection Reagent. Invert to mix. Prepare 1 hour before use to minimize assay background. Hold at room temperature. After 1 hour, add 10µl of Kinetic Enhancer per ml of Glycerol Detection Reagent. Invert to mix.

Protocol for Medium, Serum or Homogenized Tissues and Controls

1. Dilute samples in Glycerol Lysis Solution to bring their glycerol concentrations below 80µM.
2. Transfer 25µl of sample, standard or control to a 96-well plate.
3. Add 25µl of Glycerol Lysis Solution, shake briefly and incubate 30 minutes at 37°C.
4. Add 50µl of Glycerol Detection Reagent to all wells.
5. Shake the plate for 30–60 seconds by hand or using a plate shaker at a low rpm.
6. Incubate the plate at room temperature for 1 hour.
7. Record luminescence on a plate-reading luminometer.
8. Calculate glycerol concentrations by comparing luminescence of samples and standards prepared under the same conditions.

Protocol for Adherent Cells or 3D Cell Cultures and Controls

1. Remove medium from cells in the 96-well plate. Wash cells twice with 100µl of PBS.
2. Add 50µl of Glycerol Lysis Solution, shake briefly and incubate 30 minutes at 37°C.
3. Dilute samples in Glycerol Lysis Solution to bring their glycerol concentrations below 80µM.
4. **Optional:** 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.
5. Add 50µl of Glycerol Detection Reagent to all wells.
6. Shake the plate for 30–60 seconds by hand or using a plate shaker at a low rpm.
7. Incubate the plate at room temperature for 1 hour.
8. Record luminescence on a plate-reading luminometer.
9. Calculate glycerol concentrations by comparing luminescence of samples and standards prepared under the same conditions.

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For additional protocol information see Technical Manual #TM599, available online at: **www.promega.com/protocols/**

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