Triglyceride-Glo™ Assay

Instructions for Use of Products J3160 and J3161.

Quick Protocol

This quick protocol is for calculating triglyceride levels in biological samples. For complete protocol information, including reagent and information on standard curve preparation, see the *Triglyceride-Glo™ Assay Technical Manual* #TM600, 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. Exercise care 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, Kinetic Enhancer and Lipoprotein Lipase on ice; all other components can be held at 22°C until use. Use reagents on the day they are prepared, only.
- **Glycerol Lysis Solution with lipase:** Add 80µl of Lipoprotein Lipase per ml of Glycerol Lysis Solution. Invert to mix.
- **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 Tissue Samples and Controls

Prepare duplicate wells for measurements with lipase (total glycerol) and without lipase (free glycerol).

1. Dilute samples in Glycerol Lysis Solution to lower their glycerol and/or triglyceride concentrations below 80µM. Include glycerol standards and a negative control (assay background). Transfer 25µl of sample, standard or control to a 96-well plate for measurements with or without lipase.
2. Add 25µl of Glycerol Lysis Solution with or without lipase, shake briefly and incubate 30 minutes at 37°C.
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 free and total glycerol concentrations by comparing the luminescence of samples and standards prepared under the same conditions (i.e., with and without lipase). Calculate triglyceride concentrations as the difference between total and free glycerol concentrations.

Protocol for Adherent Cells, 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 with or without lipase, shake briefly and incubate 30 minutes at 37°C.
3. If needed, dilute samples in Glycerol Lysis Solution to lower their glycerol concentrations below 80µM. Add 50µl of any diluted samples, standards or controls to empty wells in a 96-well assay plate.
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 free and total glycerol concentrations by comparing the luminescence of samples and standards prepared under the same conditions (i.e., with and without lipase). Calculate triglyceride concentrations as the difference between total and free glycerol concentrations.

Additional protocol information is in Technical Manual #TM600, available online at: **www.promega.com/protocols/**

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