Cholesterol/Cholesterol Ester-Glo™ Assay

Instructions for Use of Products J3190 and J3191.

Quick Protocol

This document is a quick protocol for the calculation of cholesterol and cholesterol ester levels in biological samples. For complete protocol information, including information on standard curve preparation, see the *Cholesterol/Cholesterol Ester-Glo™ Assay Technical Manual* #TM601, which is available online at: **www.promega.com/protocols/**

This protocol includes the measurement of total cholesterol (samples assayed with esterase in Cholesterol Detection Reagent) and free cholesterol (samples assayed without esterase in Cholesterol Detection Reagent). Cholesterol ester levels are calculated as the difference between total and free cholesterol.

The protocol is for a reaction with 50µl of a prepared sample and 50µl of Cholesterol Detection Reagent in a 96-well plate. The assay can be adapted to other volumes provided the 1:1 ratio of Cholesterol 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 Cholesterol Esterase 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 Cholesterol Detection Reagent, add 10µl of Reductase Substrate per ml of Cholesterol Detection Solution and mix by inversion. Cholesterol Detection Reagent is used to measure free cholesterol.
    d. To prepare Cholesterol Detection Reagent with Esterase, add 10µl of Reductase Substrate and 10µl of Cholesterol Esterase per ml of Cholesterol Detection Solution and mix by inversion. Cholesterol Detection Reagent with Esterase is used to measure total cholesterol.
2. Prepare samples, standards and controls. Prepare duplicate wells for measurements with esterase (total cholesterol) and without esterase (free cholesterol).
    a. For Medium, Serum and Homogenized Tissue Samples:
        i. Dilute samples in Cholesterol Lysis Solution to bring their cholesterol concentrations below 80µM. Transfer 25µl of sample, standard or control to a 96-well plate for measurements with or without Esterase.
        ii. Add 25µl of Cholesterol 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 Cholesterol Lysis Solution, shake briefly and incubate 30 minutes at 37°C.
        iii. If needed, dilute samples in Cholesterol Lysis Solution to bring their cholesterol 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 Cholesterol Detection Reagent with or without Esterase 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 cholesterol concentrations by comparison of the luminescence of samples and standards prepared under the same conditions (i.e., with and without Esterase). Calculate cholesterol ester concentrations as the difference between total and free cholesterol concentrations.

Additional protocol information is in Technical Manual #TM601, available online at: **www.promega.com**

