A GloMax® 96 Microplate Luminometer Method for the Caspase-Glo® 3/7 Assay

1. INTRODUCTION

The GloMax® 96 Microplate Luminometer in combination with the Caspase-Glo® Assay provides a convenient procedure for measuring caspase-3 or -7 activities. In the Caspase-Glo® Assay a proluminescent caspse-3/7 substrate contains the signature DEVD peptide sequence. Cell lysis occurs following the addition of Caspase-Glo® 3/7 Reagent. Lysed cells release Caspase-3 or -7, which cleave the DEVD substrate from the aminoluciferin. Finally, the oxidation of the luciferin by luciferase produces light, which is measured by the GloMax® 96. The amount of light produced is proportional to the amount of caspase-3 or -7 activitiy.

The ultra-sensitive GloMax® 96 enhances the detection limit of the Caspase-Glo 3/7 Assay. The GloMax® 96 detects fewer than 2 x 10^{-4} units/100 µL of purified caspase-3 using Caspase-Glo® 3/7 Substrate. Measurements are linear from 1.9 x 10-4 units/100 µL to 11 units/100 µL (R^2=0.9881) or for five orders of magnitude (Figure 1.)

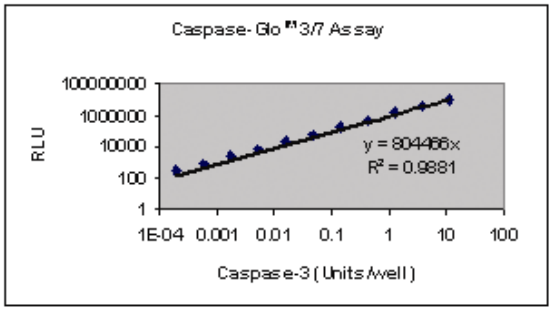

Figure 1. Caspase-Glo® Assay on the GloMax® 96 Microplate Luminometer using purified caspase-3 diluted in 25 mM HEPES buffer containing 0.1% gelatin. The sample plate incubated for 1 hour at room temperature before measurement.

2. MATERIALS REQUIRED

- GloMax® 96 Microplate Luminometer
- 96-well plates, white (E&K Scientific EK-25075)
- Caspase-Glo® 3/7 Assay kit (Cat.# G8090, G8091, G8092)
- Purified Caspase-3 (Biomol Cat.# SE-169)
- p200 pipette and pipette tips
- Plate shaker

3. PROTOCOL

3.1 Reagent Preparation

Caspase-Glo® 3/7 Substrate: Use as supplied. Store at -20°C.

Caspase-Glo® 3/7 Buffer: Use as supplied. Store at -20°C. Buffer may be thawed and stored at room temperature for 48 hours without loss of activity.

Caspase-Glo® 3/7 Reagent: Transfer the contents of one bottle of Caspase-Glo Buffer to one bottle of Caspase-Glo Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use reconstituted reagent on the same day it is prepared or store at 4°C for 3 days without loss of activity. Reagent stored at –20°C for 4 weeks will give a signal 60% of freshly prepared reagent. Reagent stored at 4°C for 4 weeks will give a signal 75% of freshly prepared reagent.

Note: Reagent stored frozen after reconstitution must be thawed below 25°C to ensure reagent performance. Mix well after thawing. The simplest method for thawing is placing the reagent in a water bath at room temperature.

3.2 Instrument Setup

3.2.1 Double-click on the GloMax® 96 icon to start the software.

3.2.2 Click on "Run Promega Protocol" from the "Welcome to GloMax® 96" dialog box.

3.2.3 Open the “Caspase-Glo” template. Enter your information into the "Experiment," "Operator," "Plate No.," and "Notes" fields in the "Main Dialog Box".

3.2.4 Click on "Options" from the "Main Dialog Box" to select the wells to be read and modify the number of runs. Once modified, click the "Apply Changes" button to return to the "Main Dialog Box".

3.3 Sample Analysis of Cell Cultures

Note: For each set of samples, prepare a negative control and a blank reaction. The blank reaction should consist of Caspase-Glo® 3/7 Reagent, vehicle and cell culture medium without cells. The negative control should consist of Caspase-Glo® 3/7 Reagent and vehicle-treated cells in cell culture medium. The term “vehicle” refers to the solvent used to dissolve the compound or protein of interest. The experimental samples should consist of Caspase-Glo® 3/7 Reagent and treated cells in cell culture medium. Positive and negative controls should be prepared for each plate during multi-batch processing. Use identical cell numbers and volumes for the assay and the negative control samples.

3.3.1 Remove 96-well plates containing cells from the incubator and allow them to equilibrate to room temperature. At the same time, allow the reconstituted Caspase-Glo® Reagent to equilibrate to room temperature.

3.3.2 Add a volume of Caspase-Glo® Reagent equal to the volume of cell culture medium. For a 96-well plate, typically 100 µL of Caspase-Glo® Reagent is added to a well containing 100 µL of cell culture medium.

3.3.3 Mix gently using a plate shaker at 300–500 rpm for 30 seconds. Incubate at room temperature for 30 minutes to 3 hours. The optimal incubation time will vary according to cell type and should be determined experimentally.

3.3.4 Insert plate into the GloMax® 96 and click on "Start" to begin assay. RLU values measured by the GloMax® 96 will appear in the Excel

spreadsheet after each selected wells has been read. If you encounter an error message, refer to the *GloMax® 96 Technical Manual* - troubleshooting guide for more information.

3.3.5 Once the measurements are complete, you can access Excel to analyze your data.

3.3.6 Remove your plate after measurement.

3.4 Sample analysis of purified enzyme

3.4.1 Prepare the blank reactions, positive controls and test samples as described in step 3.3 of this protocol.

3.4.2 Add a volume of Caspase-Glo® Reagent equal to the volume of sample material.

3.4.3 Mix gently using a plate shaker at 300–500 rpm for 30 seconds. Incubate at room temperature for 20-60 minutes. Peak luminescence occurs sooner with purified enzymes than cultured cells.

3.4.4 Insert plate into the GloMax® 96 and click on "Start" to begin assay. RLU values measured by the GloMax® 96 will appear in the Excel spreadsheet after each selected well has been read. If you encounter an error message, refer to the *GloMax® 96 Technical Manual* - troubleshooting guide for more information.

3.4.5 Once the measurements are complete, you can access Excel to analyze your data.

3.4.6 Remove your plate after measurement.

CAUTION: The lyophilized Caspase-Glo® 3/7 Substrate contains dithiothreitol (DTT) and is therefore classified as hazardous. The reconstituted reagent is not known to present any hazards as the concentration of DTT is less than 1%. However, we recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents. Promega assumes no liability for damage resulting from handling or contact with these products.

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