A 20/20^n Luminometer Method for Promega Bright-Glo™

1. INTRODUCTION

The Turner BioSystems' 20/20^n Luminometer in combination with Promega's Bright-Glo™ Assay kit provides a convenient, rapid, and sensitive procedure for quantifying gene expression. Transcriptional regulation, coupled to the expression of a luciferase reporter gene, is regularly used to study a wide range of biological events in cultured cells. Luciferase is an ideal reporter because of the absence of endogenous luciferase activity in mammalian cells, and the functional enzyme is created immediately upon translation[1,2].

The Bright-Glo™ Luciferase Assay System has been developed specifically to maximize the sensitivity of the assay reagent while providing a luminescent signal half-life of approximately 25 minutes. The light signal can be measured 2 minutes after adding assay reagents. The Bright-Glo™ Reagent is widely used in the pharmaceutical and biotechnology industries. The Bright-Glo™ Reagent is compatible with commonly used culture media for mammalian cells containing 0-10% serum (RPMI 1640, MEMα, DMEM and Ham's F12).

The superior performance of the 20/20^n combined with the effectiveness of Bright-Glo™ Reagent permits detection of very low levels of luciferase activity. The 20/20^n can detect as little as 1X10^{-20} moles luciferase enzyme (**Note:** Assay limited sensitivity) using the Bright-Glo Assay System. Measurements are linear from 1X10^{-20} to 1X10^{-12} moles luciferase or 8 orders of magnitude (Figure 1). All tests were conducted using the Bright-Glo™ Luciferase Assay Kit (Promega Catalog# E2620) and purified recombinant firefly luciferase enzyme (Promega Catalog# E1701).

Promega's Bright-Glo™ Assay

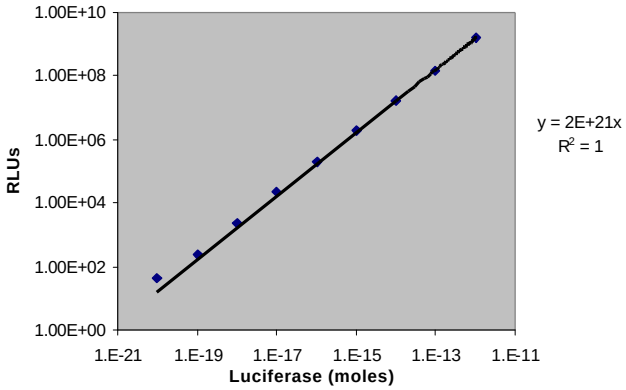

Figure 1. Bright-Glo™ Assay was perfomed on the 20/20^n Luminometer using Promega's Bright-Glo Reagent kit and recombinant luciferase.

2. MATERIALS REQUIRED

- 20/20^n Luminometer (P/N 2030-000)
- 1.5 mL microfuge tubes
- Bright-Glo™ Luciferase Assay kit (Promega Catalog #'s E2610, E2620 and E2650)
- p200 pipette and pipette tips

3. EXPERIMENT PROTOCOL

3.1 Reagent Preparation

Bright-Glo™ Substrate: Use as supplied. Store at -20°C, where it is stable for up to 6 months. The substrate may also be stored at 4°C for up to 1 month.

Bright-Glo™ Buffer: Use as supplied. Store below 25°C.

Bright-Glo™ Reagent: Transfer the contents of one bottle of Bright-Glo™ Buffer to one bottle of Bright-Glo™ Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use

reconstituted reagent on the same day it is prepared or aliquot into working volume and store at -20°C for up to 2 weeks.

Note: The temperature of the Bright-Glo™ Reagent should be held constant at room temperature while quantifying luminescence since luciferase activity is temperature dependent. 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 Turn ON the 20/20^n. A 5 minute warm up period is recommended, but not necessary.

3.2.2 Touch "Run Promega Protocol" from the "Protocols" menu.

3.2.3 Select "BrightGlo" from the list of Promega protocols. The Parameters screen appears next with pre-programmed settings that are optimized for the Bright-Glo Assay.

3.2.4 Touch OK to go to the Home Screen.

3.3 Sample Analysis

3.3.1 Remove the cell cultures from the incubator.

Note: For maximum reproducibility, equilibrate cell cultures to room temperature before adding reagent.

3.3.2 Add a volume of the Bright-Glo™ Reagent equal to that of the culture medium.

3.3.3 Wait a minimum of 5 minutes to allow for sufficient cell lysis. Then transfer the sample to a 1.5 mL microifuge tube for analysis.

3.3.4 Insert the tube into the 20/20^n and touch “Measure Luminescence” to begin measurement.

4. REFERENCES

1. Ow, D.W. *et al.* (1986) Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. *Science* 234, 856—9.

2. De Wet, J.R. *et al.* (1987) Firefly luciferase gene: structure and expression in mammalian cells, *Mol. Cell. Biol.* 7, 725—37.

5. ABOUT PROMEGA CORPORATION

Bright-Glo is a trademark of Promega Corporation and is registered with the U.S. Patent and Trademark Office. Orders for Promega's products may be placed by:

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6. ABOUT TURNER BIOSYSTEMS

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CAUTION: The lyophilized Bright-Glo™ 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 and Turner BioSystems assume no liability for damage resulting from handling or contact with these products.