A 20/20^n Luminometer Method for Promega Dual-Luciferase® Reporter (DLR) Assay

1. INTRODUCTION

The Turner BioSystems 20/20^n Luminometer in combination with Promega's Dual-Luciferase® Reporter (DLR) 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 Dual-Luciferase® Reporter (DLR) Assay System contains two different luciferase reporter enzymes that are expressed simultaneously in each cell. Typically, the experimental reporter is correlated with the effect of specific experimental conditions, while the activity of the co-transfected "control" reporter gene provides an internal control, which serves as the baseline response. Normalizing the experimental reporter gene to the activity of an internal control minimizes the variability caused by differences in cell viability and transfection efficiency. Thus, dual reporter assays allow more reliable interpretation of the experimental data by reducing extraneous influences.

The experimental and control luciferase enzymes used in the Dual-Luciferase® Reporter (DLR) Assay have distinct evolutionary origins. The firefly luciferase and the *Renilla* (sea pansy) luciferase can discriminate between their respective bioluminescent substrates and do not cross-activate.

The firefly and *Renilla* substrates have been developed specifically to maximize the sensitivity of the assay reagent. This system is widely used in life science research because of the superior light generation and high signal to noise ratio. Dual-Luciferase® Reporter (DLR) reagents are compatible with commonly used culture media for mammalian cells including RPMI 1640, MEMα, DMEM, and Ham's F12. These reagents are designed for use with the Passive Lysis Buffer that comes with the kit and is available separately from Promega (Catalog # E1910).

The sensitivity and wide dynamic range offered by the 20/20^n Luminometer make it highly suited for the Dual-Luciferase® Reporter (DLR) Assay application. The 20/20^n software facilitates a quick set-up for the Dual-Luciferase® Reporter Assay with a pre-installed template. The internal automatic injectors are easy to use and completely accessible.

The 20/20^n Luminometer can detect as little as $1X10^{-21}$ moles firefly luciferase enzyme using Luciferase Assay Reagent II (LAR II). All tests were conducted using purified recombinant firefly luciferase enzyme (Promega Catalog # E1701) and purified *Renilla* recombinant enzyme (Chemicon Catalog # 4400).

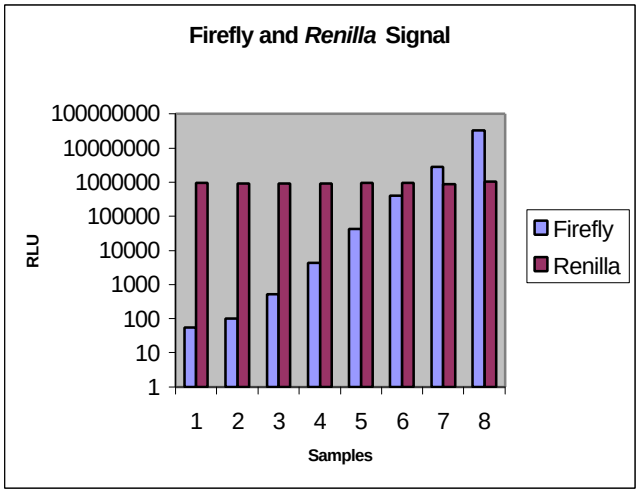

Figure 1. *DLR assay was performed on the 20/20^n Luminometer using Promega's Dual-Luciferase® Reporter Assay System with recombinant firefly luciferase (1x10^{-21} moles to 1x10^{-14} moles) and Renilla luciferase (1x10^{-16} moles).*

2. MATERIALS REQUIRED

- 20/20^n Luminometer (P/N 2030-002)
- Microfuge Tube Holder
- 1.5 mL Microfuge Tubes
- Promega Dual-Luciferase Reporter® Assay kit (Promega Catalog #E1980)
- p200 pipette and pipette tips
- p20 pipette and pipette tips

3. EXPERIMENT PROTOCOL

3.1 Reagent Preparation

Luciferase Assay Buffer II and Luciferase Assay Substrate: Use as supplied. Store at -20°C, where it is stable for up to 6 months. The Luciferase Assay Substrate may also be stored at 4°C for up to 1 month.

Transfer the contents of one bottle of Luciferase Assay Buffer II into one vial of Luciferase Assay Substrate. Mix by inversion until the substrate is thoroughly dissolved. Use reconstituted Luciferase Assay II Reagent (LAR II) on the same day it is prepared, or aliquot into working volume and store at -20°C for up to 1 year.

Stop & Glo® Substrate and Stop & Glo® Buffer: Use as supplied. Store below -20°C.

Stop & Glo® Buffer Substrate Solvent: Use as supplied. Store below 25°C.

To make Stop & Glo® Reagent, dilute the 50x Stop & Glo® Substrate to 1x concentration using Stop & Glo® Buffer in a glass or siliconized polypropylene tube. Mix by inversion. Use reconstituted Stop & Glo® Substrate on the same day it is prepared or store at -20°C for up to 2 weeks.

Passive Lysis Buffer: To make 1x Passive Lysis Buffer, dilute the 5x Passive Lysis Buffer in deionized water. Store below 25°C.

Note: The temperature of the Luciferase Assay Buffer II and Stop & Glo® Buffer 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 "DLR-2-INJ" from the list of Promega protocols. The “Parameters” screen appears next with pre-programmed settings that are optimized for the Dual Luciferase Reporter Assay with two automatic injectors.

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 Prepare the injectors. Place the inlet tubing for injector 1 into the bottle of LAR II. Place the inlet tubing for injector 2 into the bottle of Stop & Glo® Reagent. Place a waste container underneath the injector tips.

3.3.3 Touch "Inj Func" and prime both injectors.

Note: Do not switch injectors. Residual Stop & Glo® Reagent will quench the firefly luciferase reporter activity. It is recommended to dedicate the injector 2 for Stop & Glo® Reagent and injector 1 for LAR II.

3.3.4 Add 20 µL of your sample into a microfuge tube.

3.3.5 Place the microfuge tube into the tube holder and close the lid.

3.3.6 Touch "Measure Luminescence " to begin measurement. The 20/20^n will automatically inject 100 µL of LARII into the sample. After a 2-second delay, the 20/20^n will measure the firefly signal over a 10-second integration period. Then, the 20/20^n will automatically inject 100 µL of Stop & Glo® Reagent into the microfuge tube. After a 2-second delay, the 20/20^n will measure the *Renilla* signal over a 10-second integration period.

3.3.7 Repeat 3.3.6 for each sample.

3.3.8 Once all measurements are complete, flush the injectors with deionized water, followed by 70% ethanol. Perform a final flush with deionized water and allow the water to remain in the injector system.

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.

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Phone: (800) 356-9526
Fax: (800) 356-1970
E-mail: custserv@promega.com

Mailing Address:

Promega Corporation
2800 Woods Hollow Rd.
Madison, WI 53711
USA

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Fax: (408) 737-7919

Web Site: www.turnerbiosystems.com

E-Mail: sales@turnerbiosystems.com

Mailing Address:

Turner BioSystems, Inc.
645 N. Mary Avenue
Sunnyvale, CA 94085 USA

CAUTION: The lyophilized Luciferase® Assay 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.