

## Dual-Luciferase<sup>®</sup> Reporter (DLR<sup>™</sup>) Assay System

### Implementation on Tecan's Infinite<sup>®</sup> M1000 Multimode Reader



## Introduction

### Reporter Gene Assay

In recent years genetic reporter systems have greatly influenced analysis and understanding of gene expression, gene regulation and cellular responses in both eukaryotic and prokaryotic cells.

A genetic reporter system consists of a promoter or a genetic element under analysis joined to a reporter gene in an expression vector. Expression of the reporter protein can be accomplished by measuring the protein itself or the enzymatic activity of the protein.

Enzymatic assays are very sensitive, because only a small amount of the reporter enzyme is needed to generate the reaction product. Luciferase enzymes from a variety of organisms have become popular in reporter systems due to their inherent sensitivity and ease in measurement.

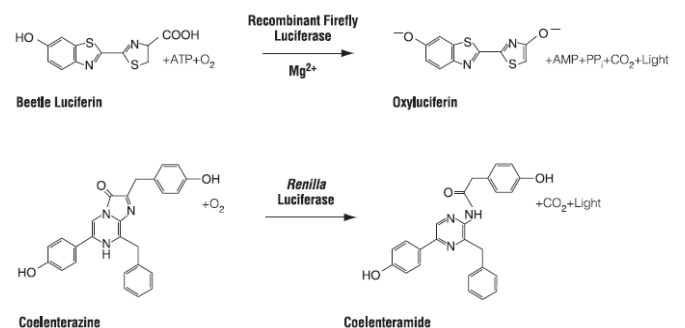
### Assay Principle

The Dual-Luciferase<sup>®</sup> Reporter Assay System from Promega Corp. is based on assaying two luciferase activities.

One reporter can be used to measure the response according to the experimental question; this reporter is usually called the "experimental" reporter. The second reporter serves as an internal control to normalize the data obtained from the experimental reporter.

The firefly luciferase from the firefly beetle (*Photinus pyralis*) is a 61 kDa monomeric enzyme catalysing the oxidation of luciferin while emitting light at about 560 nm. The smaller, also monomeric, Renilla luciferase (31 kDa) from sea pansy (*Renilla reniformis*) oxidizes coelenterazine and emits light centered about 480 nm.

According to the experimental set up, the firefly or the Renilla luciferase can be used as experimental or control reporter respectively.



**Figure 1:** Firefly and Renilla luciferase reactions

For the Dual Luciferase Reporter Assay, the activity of the firefly luciferase and the Renilla luciferase are determined sequentially. Therefore, in each well of a micro plate to be

analysed 100 µl of the firefly luciferase reagent (LAR II) is injected into a well containing a sample cell lysate. The light output over 10 sec is measured, and then 100 µl of the reagent for the second luciferase (Stop & Glo® Reagent) is injected and again the light output is measured. The second reagent stops (quenches) the first reaction and delivers the substrate for Renilla luciferase reaction.

This note describes the Dual Luciferase Reporter Assay performance using Tecan's Infinite M1000 multimode reader equipped with a two channel injector.

## Material and Methods

### Instruments

- Tecan Infinite® M1000 premium Quad4 Monochromators™ multimode microplate reader, equipped with a two channel injector and a luminescence module

### Microplate

- 96 well flat bottom white Polystyrol microplates (Corning®, NY, USA)

### Reagents

- Dual-Luciferase® Reporter Assay System (Promega Corp., USA)
- QuantiLum® Recombinant Firefly Luciferase (Promega Corp., USA)
- Recombinant Renilla Luciferase (LUX Biotechnology, UK)

### Assay Procedure

1. Thaw the reagents and prepare Luciferase Assay Reagent (LAR II) Reagent, Stop&Glo Reagent (S&GR), and 1x PLB containing 1 mg/ml gelatine (1xPLB/G) according to the manufacturers instructions and let equilibrate to room temperature.
2. While preparing the reagents switch on the Infinite M1000 instrument and load the i-Control based DLR measurement script and heat the instrument for about 30 min to the temperature the assay should be performed using the `Heating` feature.
3. Prime injector A with LARII and injector B with S&GR.
4. For quenching experiments dilute recombinant firefly luciferase in PLB/G and add 20 µl to each well of a row of micro well plate.
5. For experiments showing the firefly and Renilla luciferase signal independence, recombinant firefly and Renilla luciferase is diluted in PLB/G and mixed to achieve 1:10 and 10:1 molar ratios for firefly and renilla luciferase, respectively.
6. For each molar ratio experiment 20 µl of the resp. enzyme mix is pipetted in two rows (24 wells) of a micro well plate.
7. Place the micro well plate into the reader and start measurements.

### Measurement Parameters and Settings

Measurement Parameter	Instrument Setting
Plate	COS96fw
Mode	Luminescence well wise
Injector A	100 µl 200 µl / sec refill after injection
Wait	2 sec
Label 1, integration time	10 sec
Injector B	100 µl 200 µl / sec refill after injection
Wait	2 sec
Label 2, integration time	10 sec

**Table 2:** Dual Luminescence Reporter assay measurement parameter and instrument settings for Infinite M1000 and two channel injector

### Data Analysis

The experimental data, as well as the measurement parameters were loaded automatically by the i-Control software to Microsoft Excel for further analysis.

## Results and Discussion

The measurement was performed on Tecan's Infinite M1000 instrument using 96 well white micro plates. The results of the quenching experiments shown in Table 1 demonstrate the efficient quenching of the firefly luciferase reaction.

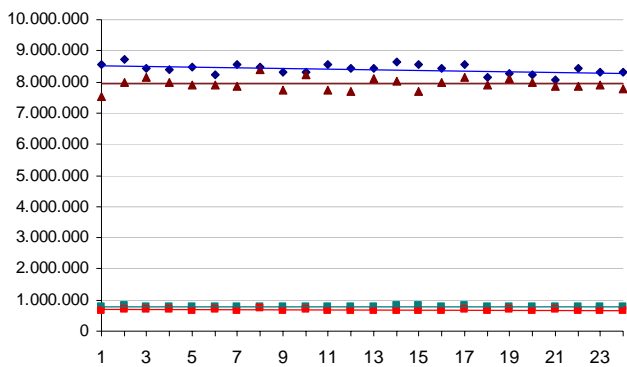
Sample	RLU Firefly Luciferase	RLU S&G	Quenching factor
1	13999329	327	42811
2	14378278	142	101255
3	14247255	76	187464
4	14372583	89	161490
5	14665982	582	25199
6	14544370	82	177370
7	14353459	581	24705
8	14399231	232	62066
9	14421364	766	18827
10	14656389	67	218752
11	14297633	138	103606
12	13920880	103	135154
<b>Average</b>	<b>14242872</b>		<b>104892</b>

**Table 1:** Exemplified data from an average quenching experiment of the Firefly Luciferase. Firefly Luciferase reaction was quenched by adding 100 µl Stop&Glo reagent. Quenching was calculated after blanking determined RLU values.

To demonstrate the consistency of the measurement of the two luciferase reactions different enzyme concentrations and mixes were tested.

Figure 2 shows a graphical summary of the firefly and Renilla luciferase light output when mixed in different molar ratios (1:10 and 10:1 resp.) and measured sequentially.

There was no interference of the light output of the two different enzymes observed and furthermore it could be shown that the signals measured over 24 wells are very consistent, with coefficients of variation (CV) of about 1.4 – 2.7 %.



**Figure 2:** Firefly and Renilla luciferase signal independence. Firefly and Renilla luciferase were mixed to achieve 1:10 and 10:1 molar ratios, respectively and RLUs were measured. Firefly (♦) and Renilla (■) luciferase 10:1; and firefly (■) and Renilla (▲) 1:10.

## Conclusion

This technical note describes the successful performance of Promega's Dual Luciferase Reporter Assay on the Tecan Infinite M1000 multimode reader.

For performing the Dual Luciferase Reporter Assay, some precautions must be taken which can be easily achieved with Tecan's Infinite M1000 multimode reader.

As is true for many enzymatic reactions, the luciferase reaction is temperature dependent. Therefore it is advisable to let the instrument equilibrate for a minimum of 30 minutes to the temperature the assay reaction is performed. This can be achieved by the instrument feature `Heating` to obtain a homogenous temperature during the measurement within the instrument.

Because of the high sensitivity of luciferase assays, proper instrument maintenance is a critical issue. Before running a Dual Luciferase Reporter assay the injectors should be cleaned very carefully. We suggest removing all the liquid within the tubing system before priming the injectors with the assay reagents to prevent reagent dilution or contamination.

After performing the assays it is recommended to clean the injectors and tubing first with distilled water and then with 70 % Ethanol for 30 min. This procedure efficiently removes the Stop&Glo reagent, which has a reversible adsorption to some kinds of plastic material. Tecan injector systems use high quality Teflon tubings, which show minimal adsorption of reagents.

Finally, it must be mentioned that some micro plates have considerable auto luminescence depending on the material used for manufacturing. Therefore, micro plates should be tested for auto luminescence before starting experiments. For plate with high auto luminescence the plate must be dark adapted to ensure high quality results.

## Acknowledgement

We would like to thank Laurie Engel and Kevin Kopish from Promega Corp. for their helpful discussions and excellent support.

## Literature

(1) Dual-Luciferase<sup>®</sup> Reporter Assay System, Instructions for use (Promega Corporation, Madison, WI, USA)

## List of abbreviations

PLB	Passive Lysis Buffer
PLB/G	Passive Lysis Buffer with gelatine (1 mg/ml)
RLU	Relative Light Units
S&GR	Stop&Glo Reagent

October 2008

Tecan Group Ltd. makes every effort to include accurate and up-to-date information within this publication, however, it is possible that omissions or errors might have occurred. Tecan Group Ltd. cannot, therefore, make any representations or warranties, expressed or implied, as to the accuracy or completeness of the information provided in this publication. Changes in this publication can be made at any time without notice. All mentioned trademarks are protected by law. For technical details and detailed procedures of the specifications provided in this document please contact your Tecan representative. This brochure may contain reference to applications and products which are not available in all markets. Please check with your local sales representative.  
© 2008, Tecan Trading AG, Switzerland, all rights reserved. Tecan is in major countries a registered trademark of Tecan Group Ltd., Männedorf, Switzerland

Tecan is in major countries a registered trademark of Tecan Group Ltd., Männedorf, Switzerland.  
Infinite is in major countries a registered trademark of Tecan Group Ltd., Männedorf, Switzerland.  
DLReady is a trademark of Promega Corporation, Madison, WI, USA. Dual-Luciferase, QuantiLum and Stop & Glo are registered trademarks of Promega Corporation, Madison, WI, USA. Corning is a registered trademark of Corning, USA.

**Austria** +43 62 46 89 33 **Belgium** +32 15 42 13 19 **China** +86 10 5869 5936 **Denmark** +45 70 23 44 50 **France** +33 4 72 76 04 80  
**Germany** +49 79 51 94 170 **Italy** +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 174 **Portugal** +351 21 000 82 16  
**Singapore** +65 644 41 886 **Spain** +34 93 490 01 74 **Sweden** +46 31 75 44 000 **Switzerland** +41 44 922 89 22 **UK** +44 118 9300 300  
**USA** +1 919 361 5200 **ROW** +41 44 922 8125