

Dual-Luciferase[®] Reporter Gene Assay

Luminescence measurements with Tecan Infinite[™] M 200



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[®] 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.

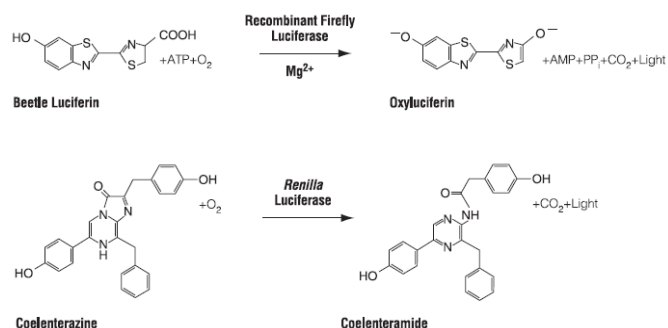


Figure 1: Firefly and renilla luciferase reactions

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™ M 200 multimode reader equipped with a two channel injector.

Material and Methods

Instrument

- Tecan Infinite M200 micro plate reader (Tecan Austria, Austria)

Microplates

- 96 well flat bottom white Polystyrol micro plates, Lumitrac 200 (Greiner Bio-one, Germany)

Reagents

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

Assay procedure

1. Thaw the reagents and prepare Luciferase Assay Reagent II (LARII), Stop&Glow 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 M 200 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. For each molar ratio experiment 20 µl of the resp. enzyme mix is pipetted in two rows (24 wells) of a micro well plate.
6. Place the micro well plate into the reader and start measurements.

Measurement Parameters

Parameter	Setting
Plate	[GRE96fw]
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 for Infinite M200

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 M 200 instrument using 96 well white micro plates. Table 1 shows the efficient quenching of the firefly luciferase reaction.

Sample	Firefly Luciferase	S&G	Quenching
1	5,508,185	74	74,435
2	5,589,571	96	58,225
3	5,540,906	65	85,245
4	5,641,428	94	60,015
5	5,668,053	92	61,609
6	5,522,657	81	68,181
7	5,521,004	84	65,726
8	5,504,265	72	76,448
9	5,594,167	71	78,791
10	5,515,535	90	61,284
11	5,432,539	86	63,169
12	5,375,449	89	60,398
Average	5,534,480		67,794
CV (%)	1.48		

Table 1: Quenching of the Firefly Luciferase. Firefly Luciferase reaction was quenched by adding 100 µl Stop&Glow reagent. Quenching was calculated after blanking determined RLU values.

Figure 2 shows the 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 observed and furthermore it could be shown that the signals measured over 24 wells are very consistent, with coefficients of variation of about 1.5 – 2.7 %.

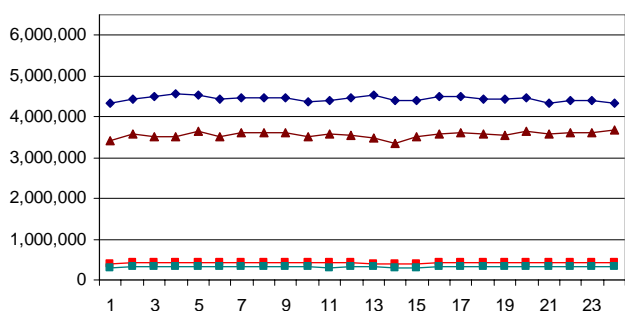


Figure 2: Firefly and Renilla luciferase signal independence. Firefly and Renilla luciferase were mixed to achieve 1:10 and 10:1 molar ratios, respectively and RLUs were measured. Firefly (♦) and Renilla (■) luciferase 10:1; and firefly (■) and Renilla (▲) 1:10.

Conclusion

This technical note describes the successful performance of Promega’s Dual Luciferase Reporter Assay on the Tecan Infinite M 200 multiple mode detection reader.

For performing the Dual Luciferase Reporter Assay, some precautions must be taken which can be easily achieved with Tecan’s Infinite M 200 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 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&Glow 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 for ensure quality results.

Acknowledgement

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Literature

(1) Dual-Luciferase® Reporter Assay System, Instructions for use (Promega Corp., MA)

List of abbreviations

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

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