Marker Gene Technologies, Inc

Product Information Sheet

RubyGlow™Glo-Gal™ β-Galactosidase Assay Kit

Product M1968

Marker Gene Technologies, Inc.
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MGT

RubyGlow™ Glo-Gal™ β-Galactosidase Assay Kit (Product M1968)

NOTE: The following information is given as a viable methodology for use of the RubyGlow™ Glo-Gal™ β-Galactosidase Assay Kit. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

I. OVERVIEW

One of the most common reporter genes used in molecular biology applications is the *E. coli lacZ* gene that codes for an active subunit of β-galactosidase *in vivo*. Since this enzyme is generally absent in normal mammalian, yeast, some bacterial and even plant cells, it can be detected at very low levels. The enzyme also has a wide substrate specificity that allows monitoring of *lacZ* expression (and therefore co-expressed genes or promoter efficiency) routinely, to the point of detection of as few as 5 copies of β-galactosidase per cell by FACS analysis.

Although chromogenic assays of β-galactosidase activity (i.e. X-Gal) have found extensive use as do the application of the fluorogenic substrates (for example FDG) detection of *lacZ* activity using luminescence can give even greater levels of sensitivity.

By coupling the β-Gal assay to the highly sensitive luciferase enzyme *lacZ* activity can be measured quickly and easy down to extremely low levels of expression. The sensitive Glo-Gal™ substrate is cleaved first by the β-galactosidase enzyme and then in the presence of ATP luciferase acts to produce the light output.

The luciferase enzyme provided in the kit has been genetically engineered to produce a red light emission (λ_{max} = 619 nm). This property allows the use of this kit with other luciferases, producing, for example, green emission wavelengths. For more information about these special techniques, please visit our website at www.markergene.com

This β-galactosidase detection kit provides reagents and protocol to perform up to 200 (2 x 96 well microtiterplate) assays. Complete details of the protocol are provided below. If you have further questions or need help with the assays, please feel free to write us at techservice@markergene.com or telephone our technical assistance staff, toll-free at 1-888-218-4062.

II. MATERIALS

A.) 1 Cell Lysis Buffer. 2 x 10ml

B.) 2 Reaction Buffer. 2 x 10ml

C.) 3 Glo-Gal™ Substrate. 2 x 15mg

D.) 4 RubyGlow™ Red Luciferase. 2 x 0.5mg

Storage and Handling: Reconstituted substrates should be handled with care, kept cold (ice-bath) when not in use, and stored frozen (-20^{o}C), long term storage of reconstituted substrate is not suggested. In case of contact with skin or eyes wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. Use in drug or manufacturing processes is strictly prohibited. Please contact us for information on use or licensing.

III. Protocol

It is recommended that a calibration curve be prepared using known concentrations of purified β-galactosidase enzyme in the approximate concentration range of the unknown analyte.

1.) Cell Lysate samples are prepared as follows: Adherent or non-adherent cells are grown to 70-80% confluency using standard tissue culture conditions. The media is removed from the cell samples by suction or mild centrifugation, and the cells washed with sterile PBS (50-100uL per well) and the PBS removed as above. Cell Lysis Buffer 1 is added to the cells (100uL/well). Cells are incubated at 37°C for 30 mins. Lysis buffer should also be added to empty wells in triplicate to act as a blank.

2.) Prepare assay solution by reconstituting 1 vial of Glo-Gal™ Substrate 3 and 1 vial of RubyGlow™ Red Luciferase 4 in 10ml of Reaction Buffer 2. It is best to transfer 10ml of Reaction Buffer 2 to vial of Glo-Gal™ Substrate 3 and then transfer 1ml of this to reconstitute the RubyGlow™ Red Luciferase 4 and then transfer this back to the Glo-Gal™ Substrate 3 vial.

3.) To each well containing unknowns and standards add 100uL of the prepared assay buffer.

4.) Incubate for 0-10 minutes then read luminescence on a luminometer equipped plate reader.

5.) Luminescence can be read over time in order to calculate enzyme kinetics.

Note (1.) It is best to use the prepared assay solution immediately; extended storage of this is not recommended.
Note (2.) Exact incubation time should be optimized for the assay being performed, alternatively where available, kinetic measurements can be taken and optimum values used.

IV. Example Data

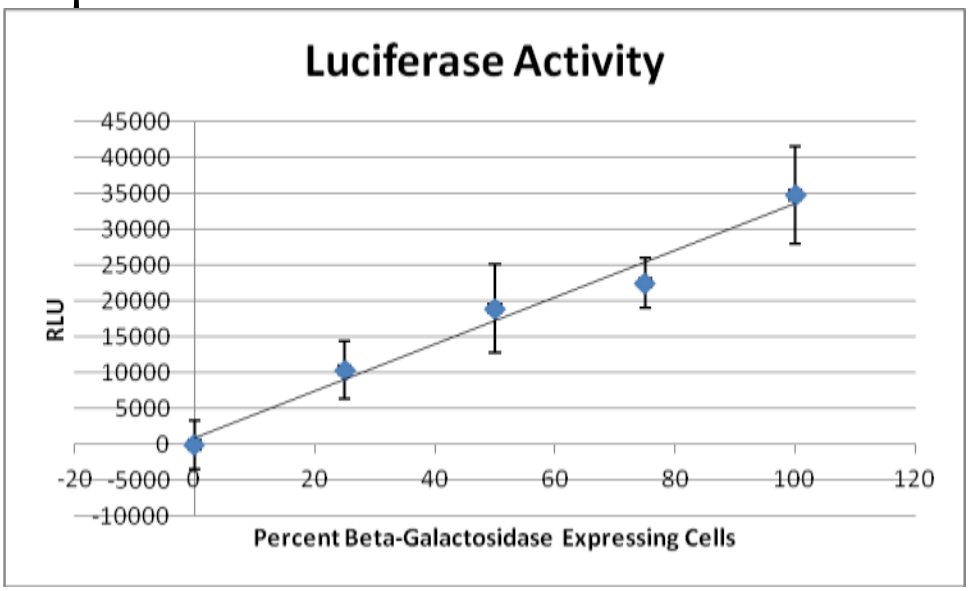

Figure 1: NIH3T3 and Cre-Bag 2 (β-Gal positive) cells were harvested and counted. Cell suspensions were prepared containing 100%, 75%, 50%, 25% and 0% NIH3T3 and plated in a 96 well plate in triplicate and allowed to grow overnight. The next day 50uL Cell Lysis Buffer was applied and incubated 30mins. 50uL of assay solution prepared as above was added to all cell-containing wells. 50uL of assay solution was added to the 3 wells containing only Cell Lysis Buffer. Plate was incubated 10mins at RT and then read in a Tecan Infinite M200 Pro plate reader.

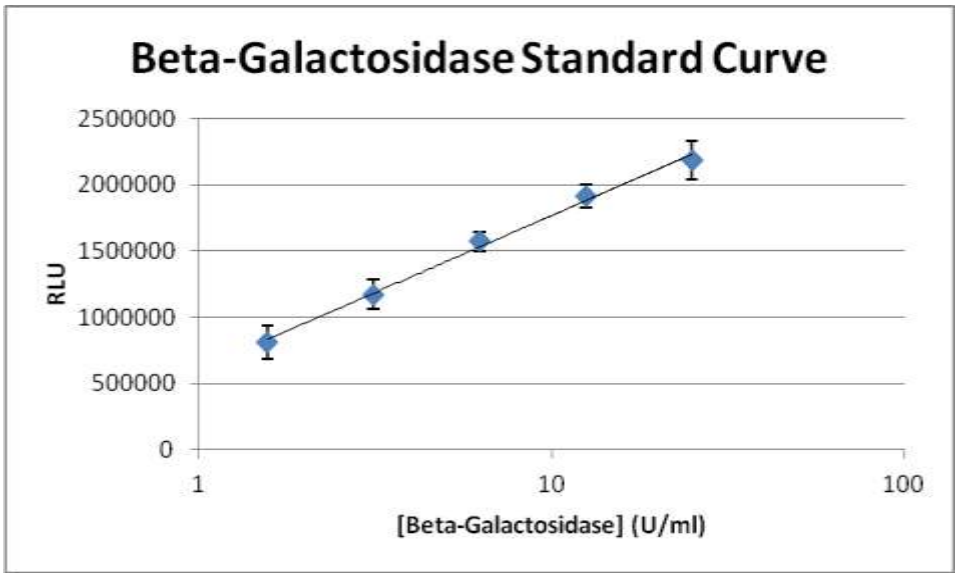

Figure 2: Purified beta-Galactosidase was made into a 25U/ml solution in Cell Lysis Buffer and serially diluted down to 1.5U/ml. 50uL of these solutions was pipetted in triplicate into a 96 well plate with lysis buffer blanks. 50uL of prepared assay solution was added and the plate read immediately in a Tecan Infinite M200 Pro plate reader.

M1968 KIT CONTENTS			
DESCRIPTION	QUANTITY	PART NO.	STORAGE
REAGENTS			
1 **Cell Lysis Buffer**	2 x 10mL	1968-001	C
2 **Reaction Buffer**	2 x 10mL	1968-002	C, R
3 **Glo-Gal™ Substrate**	2 x 15mg	1968-003	F, R
4 **RubyGlow™ Red Luciferase**	2 x 0.5mg	1968-004	F, R

Notes: F=store at or below -20°C; RT=store at room temperature;
C=store cold (4°C); L=light sensitive; FL=flammable;
R=read protocol instructions carefully prior to use.

REFERENCES

1.) Fukuda S, Murakami S, Tatsumi H. Recombinant firefly luciferase and their application for the detection of microorganisms. Bioluminescence & Chemiluminescence, Proceedings of the International Symposium, 11th, Pacific Grove, CA, United States, Sept. 6-10, 2000 (2001), Meeting Date 2000, 285-288.

2.) Yang X, Janatova J, Andrade JD. (2005),"Homogeneous enzyme immunoassay modified for application to luminescence-based biosensors." Anal. Biochem. 336(1), 102-107.

3.) Bronstein I, Fortin J, Stanley PE, Stewart GS, Kricka LJ. (1994) "Chemiluminescent and bioluminescent reporter gene assays." Anal. Biochem. 219: 169.

4.) Geiger R, Schneider E, Wallenfels K, Miska W. (1992) "A new ultrasensitive bioluminogenic enzyme substrate for beta-galactosidase." Biol. Chem. Hoppe Seyler. 373(12):1187-91.

CONTACT AND SUPPORT

For questions or comments on this or any product from Marker Gene Technologies, Inc., you may contact us by phone or via our website. We welcome customer feedback and we make every effort to improve our products based on input from our clients.

To ask a question or make a comment or suggestion, you can call us at **1-888-218-4062** or fax to **541-342-1960**.

For more information on our products and services, please visit our website at www.markergene.com, where you can find:

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We want to thank you for your purchase and hope that you will continue to order from Marker Gene Technologies, Inc.

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