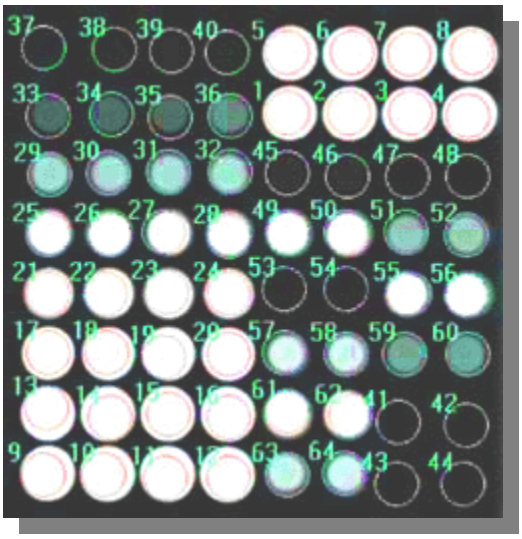
PRODUCT INFORMATION SHEET



MARKERGENE™ CHEMILUMINESCENT lacZ β-GALACTOSIDASE DETECTION KIT

(Product M0855)

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MarkerGene™ Chemiluminescent lacZ β-Galactosidase Detection Kit

(PRODUCT M0855)

NOTE: The following information is given as a viable methodology for use of the MarkerGene™ Chemiluminescent *lacZ* β-Galactosidase Detection Kit. The user may determine their own best methods for use dependent on the specific conditions present in their experiment.

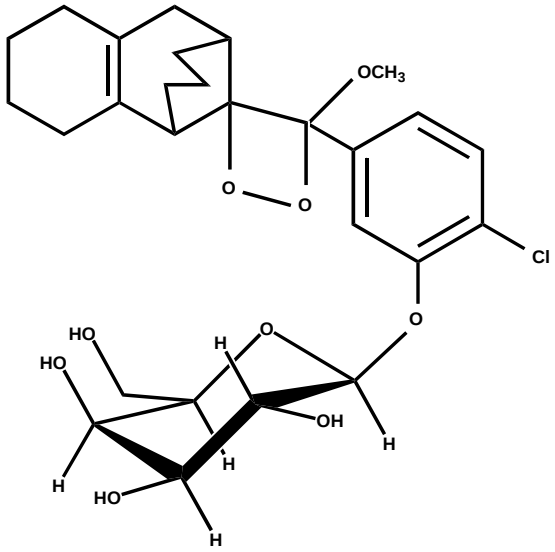
I. OVERVIEW

Reporter genes are widely used as "markers" for analysis in gene regulation and localization, as well as for analysis of mutation altered genes. Expression of reporter genes can be measured by immunological assay, biochemical activity assay or by histochemical staining of cells or tissues. 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, and since the enzyme has a wide substrate specificity, monitoring *lacZ* expression (and therefore co-expressed genes or promoter efficiency) has become routine to the point of detection of as few as 5 copies of β-galactosidase.

Although chromogenic assays of ß-galactosidase activity (i.e. X-Gal) are useful, the recent application of chemiluminescent 1,2 Dioxetane substrates, which emit visible light upon enzyme catalysis, provide rapid results with very low background and high signal intensity. An enhancing solution is also provided with this kit to increase light production efficiency in solution-based assays by drawing water away from the signal production site. The Chemiluminescent lacZ β-Galactosidase Detection Kit provides all the necessary reagents, buffers, substrate, and protocols for up to 100 sensitive and quantitative lacZ β-galactosidase activity assays.

II. MATERIALS

A.) **Substrate Solution (#0855-001)**: 2.5mL 0.080mg/mL (0.15mM [(4-Methoxy)-4-(3-b-D-galactopyranosyl-4-chlorophenyl)] spiro [1,2-dioxetane-3, 13'-tricyclo[7,3,1,$0^{2,7}$]tridec2,7-ene] (AttoGlow β-Gal-π–Dioxetane) in Tris buffer plus 2.5mL Reaction Buffer containing 100mM Sodium phosphate, pH 7.5, and 1mM $MgCl_2$ (5mL total). Specific assay conditions may require a lower concentration of chemiluminescent substrate. Optimum dilutions should be determined based on individual assay conditions, as necessary.



[(4-Methoxy)-4-(3-b-D-galactopyranosyl-4-chlorophenyl)] spiro [1,2-dioxetane-3, 13'-tricyclo[7,3,1,$0^{2,7}$]tridec-2,7-ene]

A.) **Triggering Solution (#0855-002)**: Cross-linked partially water-soluble polymer (0.7g) dissolved in 100mL of Tris buffer, pH 9.6 (5 mL).

B.) **Purified β-Galactosidase Enzyme (#0855-003)**: Contains 9 units per milligram; dissolve in di-H_2O to give 1 unit per 10μL solution (i.e., dissolve 1mg in 90μL DI-H_2O).

Storage and Handling. All materials should be handled with care and stored at 4°C. Reagents should be stable for at least 6 months following purchase. High background readings for blank samples may indicate decomposition.

III. CELLULAR ASSAY CONDITIONS

It is recommended that measurements be made in triplicate, if possible, and that the approximate concentration range of the chemiluminescent probe be adjusted for optimum signal and sensitivity. Previous studies have indicated that the labeling of cells is practically independent of the initial substrate concentration in the range of about 100pM - 2mM. Since light emission is time dependent, a time course for the experiments should also be generated for initial trials. The user is asked to consult with the manufacturer (or instrument manual) of the particular instrument in use for appropriate setup conditions needed for monitoring luminescence using a microplate assay format. Typical microscopic analysis can be performed using a long integration time (typically 5 min.) for photographic documentation of the light emission. Focus and aspect parameters are performed by Nomarski (visible light) prior to integration under dark conditions.

To normalize data, each cell suspension or plate is monitored at exactly the same time (i.e. 20 min.) after equilibration with the enhancer solution. The product-forming rate is dependent on the concentration of incorporated probe in the cell, but with concentrations of the probe above approximately 2µM, enzyme kinetics are typically independent of initial concentration. The working range of the assay will need to be determined for each individual experiment. Adjust working concentrations accordingly. A blank prepared with all reagents, substituting corresponding non-*lacZ*-expressing cells should be run in parallel if possible. Alternately, a blank prepared with all reagents except cells (substituting water or media) can be run to determine background readings for each experiment. Assay can also be performed with purified β-galactosidase, provided in this kit, for comparison with cellular assays, and to estimate the β-galactosidase levels in particular samples.

IV. CELLULAR PROTOCOL

1.) Healthy cells expressing the β-galactosidase gene or endogenous β-galactosidase activity are harvested from exponential growing cultures. (Note: unhealthy cells or those grown to confluence will often exhibit high background staining due to endogenous β-galactosidase activity or low pH conditions (lysosomal hydrolysis).

2.) Adherent cells are plated into 12-well plates at a concentration of approx. $1x10^5$ cells/well and grown in media (+5% FCS) under sterile, humidified conditions, 37°C, 5 % CO_2 atmosphere, overnight.

3.) Media is removed from the wells (except for blank controls) and the cells are washed with sterile PBS (1 x 500μL). PBS is removed from the wells by suction pipette.

4.) 500μL Substrate Solution (#0855-001) is added to each well. (NOTE: Alternately, Substrate Solution can be added directly to cell media, but luminescence intensity can be affected. Plates will still need to be incubated, and substrate and media will need to be removed before Triggering Solution is added).

5.) Plates containing the substrate reagent are incubated as above for 30-45min.

6.) The Substrate Solution is removed and Triggering Solution (#0855-002) (200μL/well) is added.

7.) Plates are read immediately using a luminescence plate reader, in luminescence mode with optimal dynamic range and maximum integration time (typically 1.5 to 5 sec. per sample).

8.) Readings can also be rerun at various time intervals (5, 10, 15 min.) to obtain kinetic data for luminescence. Best results are obtained between 2-10 minutes.

9.) Equivalent multiple wells are averaged. Controls are subtracted from sample well data.

10.) A standard curve is generated for purified enzyme assay trials. NOTE: A Purified β-Galactosidase Enzyme (#0855-003) sample is provided for your convenience in standardizing this assay system. However, since enzyme solutions are labile, it may be necessary to acquire and prepare a fresh purified enzyme sample before running each assay for

optimal estimation of β-galactosidase levels. Comparison with known cell lines expressing *lacZ* β-galactosidase is recommended.

11.)

12.) 11.) Relative luminescence for sample wells are compared to standard curve data, and used to determine approximate *lacZ* β-galactosidase activity in sample wells. The relative *lacZ* β-galactosidase activity for different cell lines should be determined in parallel assays, using the same conditions. Transfection efficiency can be estimated by use of a known positive control like the murine CREBAG2 cell line (ATCC CRL1858) or equivalent cell line for your particular system. The user is advised to consult the American Type Culture Collection (www.atcc.org) for a list of *lacZ* expressing cell lines.

NOTE: Clear tissue culture plates (Falcon 353503 or Corning (Costar) 3513) may exhibit some optical light transfer between wells. If possible, leaving blank wells between each sample well will reduce this "cross-over" effect, and provide better results. Another option is to remove solutions in each well of a clear tissue-culture plate and transfer to a white plate prior to luminescence measurement. Please consult the manufacturers information for the availability of white or black plates for your particular luminescence assay.

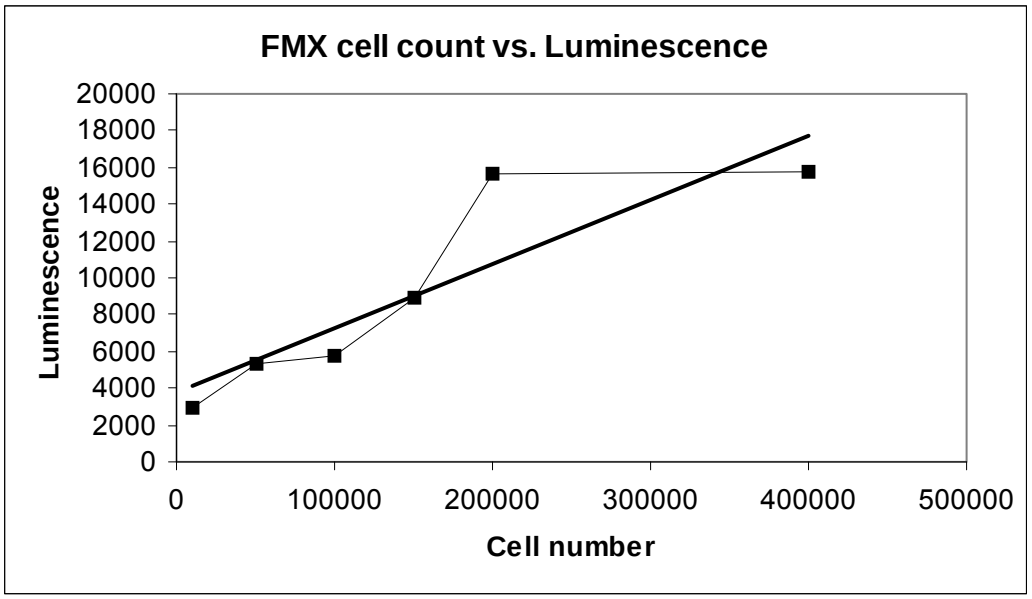


Figure 1. Assay performed with different cell numbers FMX/*lacZ* (human melanomas, retroviral transfected with lacZ).

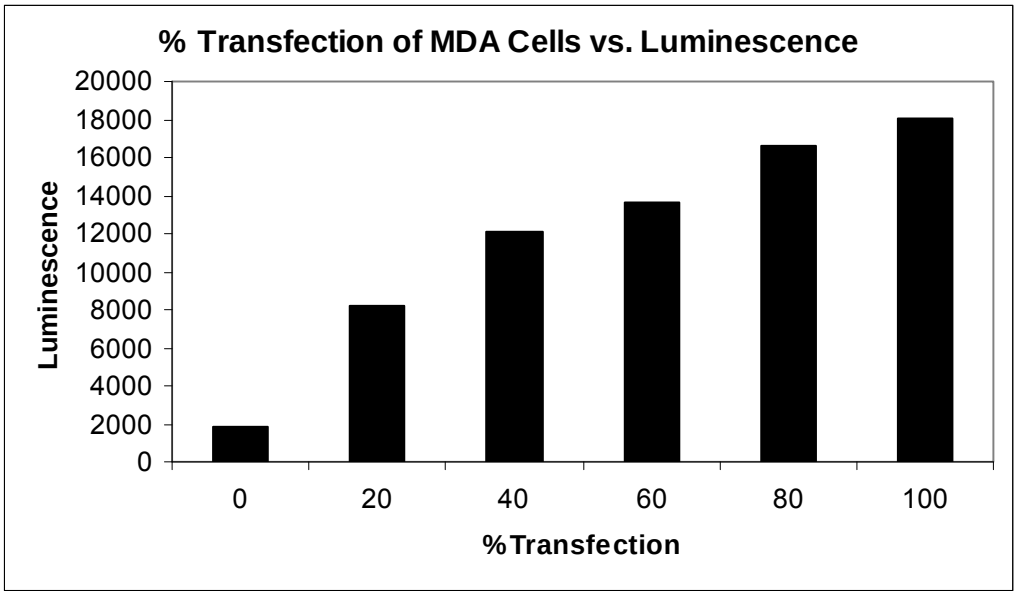


Figure 2. Assay was performed with mixed populations of MDA (human epithelial cell) and MDA/*lacZ* cells (clone created by retroviral transfection, verified to express β-gal steadily) to mimic the various percent of cell transfected. Cell luminescence was measured using a Perkin-Elmer HTS 7000 Plus UV/FL/LUM Microtiterplate reader in 12-well clear plates (a total of ~10^5cells per well).

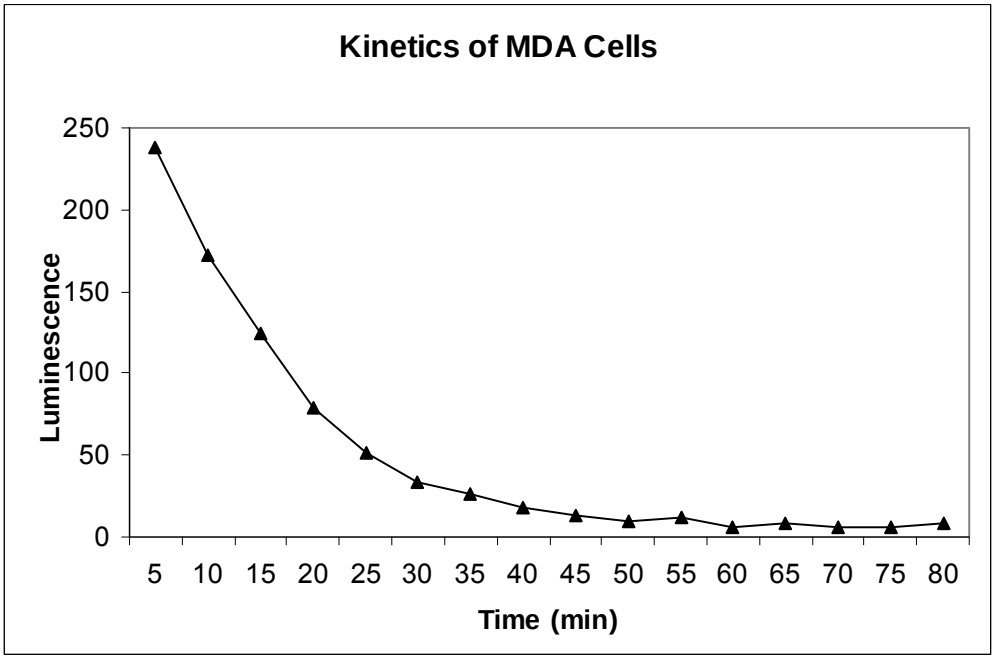


Figure 3. Assay was performed with MDA/*lacZ* (human epithelial steadily transfected cells) on 12-well clear plates (~10^5cells per well). Measurements were taken at five minute intervals using a Perkin-Elmer HTS 7000 Plus UV/FL/LUM Microtiterplate reader.

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16.) *Chemiluminescent assay for β-galactosidase activity in* E. coli *lysates; Luminescence Tropics* ***5(1):****2, (1996). Ding, L. and M. Navre.*

M0855 KIT CONTENTS			
DESCRIPTION	**QUANTITY**	**PART NO.**	**STORAGE**
SAMPLE PREPARATION			
Substrate Solution: 2.5 mL of 0.080mg/mL (~0.15mM) AttoGlow β-Gal-π–Dioxetane in Tris buffer plus 2.5mL Reaction buffer containing 100mM Sodium phosphate, pH 7.5, and 1mM $MgCl_2$	1 X 5 mL VIAL	0855-001	C,G
Triggering Solution: Cross-linked partially water-soluble polymer in Tris buffer	1 X 5 mL VIAL	0855-002	**C,G**
Purified β-Galactosidase Enzyme: dissolve in 0.9mL dH_2O to give ~1 unit per 10µL of solution	1 X 10mG	0855-003	**C,G**
DOCUMENTATION			
MSDS SHEETS	1	0855-004	N/A
PRODUCT INFORMATION SHEET	1	0855-005	N/A

Notes: F=store at or below -18°C; R=store at room temperature; C=store cold (4°C); L=light sensitive; D=store desiccated; FL=flammable; G=wear protective clothing/gloves/safety glasses when using; B=avoid breathing dust/fumes.

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**.

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