Marker Gene Technologies, Inc

Product Information Sheet

MarkerGene™ β-Glucuronidase (GUS) Reporter Gene Activity Detection Kit

Product M0877

Marker Gene Technologies, Inc.
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MarkerGene™ β-Glucuronidase (GUS) Reporter Gene Activity Detection Kit
(Product M0877)

NOTE: The following information is given as a viable methodology for use of the MarkerGene™ GUS Reporter Gene Activity 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.[1]

The β-glucuronidase (GUS) enzyme from E. coli (EC 3.2.1.31) has been well documented to provide desirable characteristics as a marker gene in transformed plants. The GUS reporter gene system has many advantages including stable expression of E. coli GUS enzyme, no interference with normal plant metabolism, and low intrinsic GUS activity in higher plants. The enzyme is also capable of tolerating amino-terminal additions, making it useful for study of plant organelle transport.[2,3,4,5]

Various β-glucuronic acid substrates are available for detection of GUS expression, all of which contain the sugar D-glucopyranosiduronic acid attached by glycosidic linkage to a hydroxyl group of a chromogenic, fluorogenic, or other detectable molecule.[5] The most widely used fluorogenic substrate for detection of β-glucuronidase activity in vitro is 4-methylumbelliferyl β-D-glucuronide (4-MUG). Upon hydrolysis by GUS, the fluorochrome 4-methylumbelliferone (7-hydroxy-4-methyl coumarin) is produced along with sugar glucuronic acid.[5] It is used in this fluorescent activity detection test.

Plants or other cell types are extracted with GUS extraction buffer containing phosphate-EDTA, pH 7.0 and detergents. The extracted β-glucuronidase hydrolyzes the 4-MUG to the fluorescent compound 4-MU (pK_a 8.2) and glucuronic acid. The reaction is stopped with sodium carbonate buffer because 4-MU exhibits maximal fluorescence at pH values above its pK_a. 4-MU can be excited at 365nm and its emission maximum is 455nm.[5]

The MarkerGene™ GUS Reporter Gene Activity Detection Kit provides reagents, buffers, substrate, and protocols for sensitive and quantitative activity assays.

II. MATERIALS

A.) Buffer Solutions.

1) GUS Extraction Buffer [1]

2) Carbonate Stop Buffer [2]: 1M Na_2CO_3.
Dilute to 0.2M Na_2CO_3 for use in the assay.

B.) GUS Assay Buffer [3]: 2mM 4-MUG (4-Methylumbelliferyl β-D-Glucuronide) in extraction buffer. Dilute 500µL of the 2mM 4-MUG solution into 9.5mL extraction buffer to yield a 0.1mM 4-MUG solution for use in the assay. Specific conditions may require higher or lower concentrations of 4-MUG, so dilute as necessary.

C.) Concentrated MU calibration stock solution [4]: 1mM 7-hydroxy-4-methylcoumarin (4-MU). Dilute 10µL of this into 10mL deionized water to make a 1µM MU stock. Store at 0-5°C protected from light. Dilute this in 0.2M carbonate stop buffer as needed to make 4-MU standard dilutions for use in extract activity calculations.

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 fluorescence readings for blank samples will indicate decomposition.

III. ASSAY CONDITIONS

1.) Plant/Cell Extraction: This procedure may be modified or replaced with a different procedure as needed. Weigh 5-50mg plant tissue, add 200-500μL of *cold* GUS Extraction Buffer[1], and grind with mortar and pestle until homogenized. Place sample in microcentrifuge tubes and centrifuge for 8 minutes at 8000rcf. Use supernatant to measure protein concentration according to, for example, Bradford (1976). Either use the plant extract immediately or store frozen at -80°C(freezer or liquid nitrogen). Do not store the extract at -20°C; enzyme activity is lost at -20°C. NOTE: For other cell types (mammalian, bacterial, yeast, etc.), cell lysis conditions may vary. Consult with literature references for information about specific lysis conditions.

2.) Performing the assay: To a 96-well microtiter plate, add the GUS Assay Buffer[3], GUS Extraction Buffer[1], and plant extract, cell lysate or blank solution (extraction buffer) as described in the table below. Allow at least four wells for each concentration of MUG (two with plant extract or cell lysate and two with extraction buffer to serve as blanks and correct for any nonenzymatic hydrolysis of MUG). Modify dilutions of MUG and/or plant extract/cell lysate and conditions if necessary. For example, if a higher MUG concentration is needed, use the GUS Assay Buffer[3] undiluted to give final [MUG] of 1.6mM, 1.2mM, 0.8mM, 0.4mM, and 0.2mM, respectively. Incubate plate at ~38°C for 10 minutes, then remove from heat and let sit at room temp. for 2-3 hours. Add 200μL 0.2M Carbonate Stop Buffer to each well. Measure fluorescence with emission and excitation filters set at 465nm and 360nm, respectively. Average the values and subtract the blank. To calculate extract activity it is necessary to perform the above assay using only one chosen concentration of MUG, for example 1.6mM, where stop buffer is added to each of four wells (two with extract and two serving as blanks) at 20 minute intervals and fluorescence of those wells with stop buffer is measured and recorded. For the zero minute test points add stop buffer to wells immediately after addition of plant extract sample and record fluorescence. Average the values for each time interval and subtract the blank. Prepare 4-MU standard[4] dilutions in Carbonate Stop Buffer[2]—concentrations of 10nM to 100nM are suitable. Include a minimum of 5 dilutions in order to plot a standard curve (10nm equals 20pmol in a 2mL cuvet). Aliquot 200-300μL of each dilution in duplicate into a microtiter plate and measure fluorescence.

Table 1. Sample assay conditions

[MUG] (Final)	GUS Assay Buffer (0.1 mM MUG)	GUS Extraction Buffer	Enzyme or Blank Solution
0.08 mM	80 μL	10 μL	10 μL
0.06 mM	60 μL	30 μL	10 μL
0.04 mM	40 μL	50 μL	10 μL
0.02 mM	20 μL	70 μL	10 μL
0.01 mM	10 μL	80 μL	10 μL

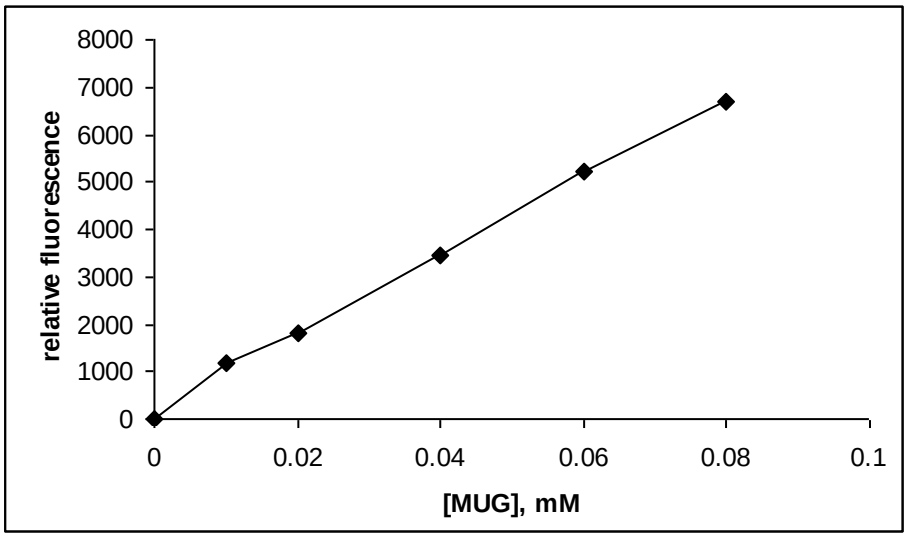

Figure 1. Assay performed with plant extract from flowers of *Arabidopsis thaliana*. Fluorescence was measured using a Perkin-Elmer HTS 7000 Plus UV/FL/LUM Microtiterplate reader.

Calculations: Plot a calibration curve of 4-MU standards fluorescence intensity (FI) vs. pmol 4-MU. Plot a curve of sample FI vs. time. Calculate FI per pmol 4-MU and FI per minute of extract sample. Calculate β-glucuronidase activity of extract in pmol 4-MU per μg protein according to equation below.

Sample calculation:

$$\text{Activity of extract (pmol MU/min/μg)} = \frac{\text{FI/min}}{\text{FI/pmol MU}} \times \frac{\text{reaction volume (μL)}}{\text{sample volume (μL)}} \times \frac{1}{\text{Vol. per test (μL)}} \times \frac{1}{\text{extract conc. (μg protein/μL)}}$$

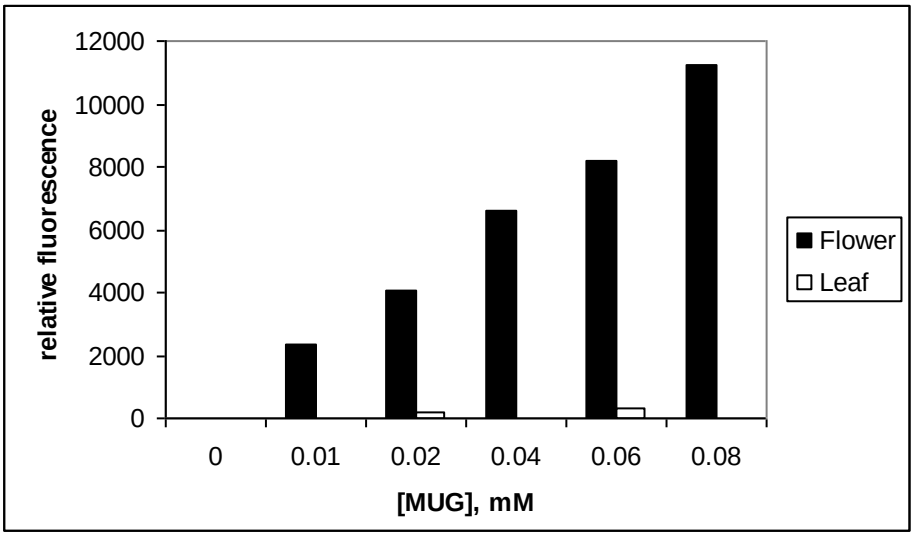

Figure 2. Comparison of GUS activity in leaf and flower extracts from *Arabidopsis thaliana.*

REFERENCES

1.) Kain SR, Ganguly S. (1996) "Uses of Fusion Genes in Mammalian Transfection." Current Protocols in Molecular Biology Vol 1, Sup 36, Ausubel FM ed (1996) p. 9.6.1.

2.) Jefferson RA, Burgess SM, Hirsh D. (1986) "beta-Glucuronidase from *Escherichia coli* as a gene fusion marker." Proc Natl Acad Sci USA. 83: 8447-8451.

3.) Jefferson RA, Kavanagh TA, Bevan MW. (1987) "GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants." EMBO J 6: 3901-3907.

4.) Kosugi S, Ohashi Y, Nakajima K, Arai Y. (1990) "An improved assay for beta-glucuronidase in transformed cells: methanol almost completely suppresses a putative endogenous for beta-glucuronidase activity." Plant Sci 70: 130-140.

5.) Gallagher SR (1992) GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression. Academic Press, Inc.

6.) Horvath BM, Magyar Z, Zhang Y, Hamburger A, Bako L, Visser RGF, Bachem CWB, Bogre L. (2006) "EBP1 regulates organ size through cell growth and proliferation in plants." EMBO J 25: 4909-4920.

M0877 KIT CONTENTS			
DESCRIPTION	**QUANTITY**	**PART NO.**	**STORAGE**
REAGENTS			
1 GUS Extraction Buffer	1 x 25 mL	0877-001	C, G
2 Carbonate Stop Buffer	1 x 25 mL	0877-002	C, G
3 GUS Assay Buffer	1 x 5 mL	0877-003	C, G
4 4-MU calibration stock solution	1 x 5 mL	0877-004	C, G
DOCUMENTATION			
MSDS Sheets	3		
Product Information Sheet	1		

Notes: F=store at or below -20°C; C=store cold (4°C); L=light sensitive; T=avoid repeat freeze/thaw; R=read protocol instructions carefully prior to use; G=wear protective clothing/gloves/safety glasses when using.

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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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