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GUS-Light™ System

Chemiluminescent Reporter Gene Assay for Detection of β-Glucuronidase in Cell Extracts

Cat. Nos. BGM100G, BGM300G, BGM2500G,
BGP100G, BGP300G, BGP2500G,
& ABG120RG

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Version 9004-C.1

I. INTRODUCTION

The Tropix® GUS-Light™ chemiluminescent reporter gene assay system is designed for rapid and sensitive detection of β-glucuronidase reporter enzyme (1) in plant or animal cell extracts. The GUS-Light™ reporter gene assay (2-4) incorporates Tropix Glucuron® chemiluminescent substrate and a light emission Accelerator. The chemiluminescent assay has a wide dynamic range, enabling detection of 60 fg to 2 ng of β-glucuronidase.

The β-glucuronidase detection assay is simple and fast. Cell extract is incubated with Reaction Buffer for 1 hour. Glucuron® chemiluminescent substrate present in the Reaction Buffer is catalytically decomposed by the enzyme. The sample is placed in a luminometer and Accelerator is added, which terminates β-glucuronidase activity and triggers the light emission, and then the light signal is measured. The amount of cell extract used should be adjusted to keep the light signal within the linear range of the luminometer. High intensity signals may saturate the photomultiplier tube of a luminometer resulting in artificially low values.

The GUS-Light™ system has been formulated for luminometers equipped with automatic injectors. Manual additions may be performed, however Accelerator addition/measurement should be performed at time intervals equivalent to Reaction Buffer addition for each sample. Reaction component volumes may be scaled down if a smaller volume injector is used. Alternate lysis buffers may be used, however we recommend that their performance be compared with GUS-Light™ Lysis Solution to ensure optimum results. A lysis buffer compatible with the luciferase assay containing 0.1 M potassium phosphate, 1 mM DTT, and 1 mg/mL BSA has been tested with equivalent performance to the GUS-Light™ system Lysis Solution.

Bacterial contamination of plant material can cause high background. Best results will be obtained with sterile preparations. Chlorophyll in concentrated samples may interfere with the chemiluminescent signal intensity. Therefore, if high levels of chlorophyll are present, several dilutions of extract should be assayed.

II. SYSTEM COMPONENTS

Shelf-life of all GUS-Light™ kit components is 1 year at 4°C.

	BG'X'100G	BG'X'300G	BG'X'2500G
microplate assays	600	1,800	15,000
Lysis Solution	70 mL	210 mL	1.75 L
Reaction Buffer Diluent	40 mL	120 mL	1 L
Glucuron® substrate	0.4 mL	1.2 mL	10 mL
Light Emission Accelerator	70 mL	210 mL	1.75 L

1. **Lysis Solution for Plant Tissue:** 50 mM sodium phosphate (pH 7.0), 10 mM EDTA, 0.1% sarkosyl, 0.1% Triton X-100 (in BGP100G, BGP300G & BGP2500G kits).

OR

Lysis Solution for Mammalian Cells: 100 mM potassium phosphate (pH 7.8), 0.2% Triton X-100 (in BGM100G, BGM300G & BGM2500G kits).

2. **Reaction Buffer Diluent:** 0.1 M sodium phosphate (pH 7.0), 10 mM EDTA.

3. **Glucuron® Substrate:** 100X concentrate.

4. **Light Emission Accelerator:** Ready to use reagent containing Emerald™ enhancer.

III. β-GLUCURONIDASE DETECTION PROTOCOL

Please read the entire Protocol and Notes sections before proceeding. Perform assays in triplicate at room temperature.

A. Preparation of Cell Extracts

1. Add DTT (to 0.5 mM) to the required volume of Lysis Solution (if desired, see Note 1).

2. Rinse cell cultures twice with PBS, or prepare a sample of plant material.

3. Add Lysis Solution to cover the cells (250 µL per 60 mm plate or 25 mg plant material).

4. Detach cells from plate by scraping (for plant material, grind tissue in a microhomogenizer).

5. Transfer the cell lysate to a microfuge tube and centrifuge for 2 min to pellet debris.

6. Transfer extracts (supernatant) to a fresh tube. Use immediately or store at -70°C.

B. Direct Lysis Procedure for Microplate Cultures

This procedure is for adherent cells growing in 96-well tissue culture-treated luminometer plates.

1. Add DTT (to 0.5 mM) to the required volume of Lysis Solution (if desired, see Note 1).

2. Rinse cell cultures once with PBS.

3. Add 10 µL of Lysis Solution to each well and incubate for 10 min.

4. Continue with Detection with Microplate Luminometers (Section D), omitting Step 3.

C. Detection with Tube Luminometers

1. Dilute Glucuron® substrate 1:100 with Reaction Buffer Diluent to make Reaction Buffer. Prepare only enough for immediate use (180 µL/tube).

2. Equilibrate Reaction Buffer and Accelerator to room temperature.

3. Transfer 2-20 µL of extract to luminometer tubes (see Note 2).

4. Add 180 µL of Reaction Buffer. Mix and incubate for 15-60 min (see Notes 3 & 4).

5. Place tubes in luminometer. Inject 300 µL of Accelerator. After a 1-2 sec delay, read the signal for 0.1-1 sec/tube.

D. Detection with Microplate Luminometers

1. Dilute Glucuron® substrate 1:100 with Reaction Buffer Diluent to make Reaction Buffer. Prepare only enough for immediate use (70 µL/well).

2. Equilibrate Reaction Buffer and Accelerator to room temperature.

3. Transfer 2-20 µL of extract to microplate wells (see Note 2).

4. Add 70 µL of Reaction Buffer. Mix and incubate for 15-60 min (see Notes 3 & 4).

5. Place microplate in luminometer. Inject 100 µL of Accelerator. After a 1-2 sec delay, read the signal for 0.1-1 sec/well.

E. Protocol Notes

1. For plant cells or tissue, add β-mercaptoethanol to Lysis Solution for Plants to a final concentration of 10 mM. For mammalian cells, add DTT to Lysis Solution to a final concentration of 0.5 mM. It should be noted that reducing agents may increase assay background, and high concentrations will decrease light emission half-life. If low background or extended half-life is critical, reducing agents should be omitted. If it must be used, adding H_2O_2 to Accelerator (to 10 mM; 0.03%) will reduce its effects.

2. The amount of extract required may vary depending on the β-glucuronidase expression level and the instrumentation used. Use 2-5 or 10-20 µL of extract for samples with high or low levels of enzyme, respectively. For experiment-to-experiment consistency, the same volume of sample should be assayed every time. Lysis Solution may be added to equalize sample volumes.

3. Incubations may be as short as 15 min (especially with high levels of expression), but the dynamic range of the assay may decrease.

4. Due to the kinetics of the assay, it is important that each well have an equal incubation time prior to measurement. Reaction Buffer should be added to samples in the same sequence that they will be measured. In addition, timing of Reaction Buffer addition should correspond to the timing of Accelerator addition/measurement.

APPENDICES

A. Preparation of Controls

Positive Control
Reconstitute lyophilized β-glucuronidase (Sigma Cat. No. G-7896) to 1 mg/mL in 0.1 M sodium phosphate (pH 7.0), 0.1% BSA. Store at 4°C. Generate a standard curve by serially diluting in Lysis Solution containing 0.1% BSA. Use 2 ng of enzyme as an upper detection limit.

Negative Control
Assay a volume of mock-transfected extract equivalent to that of experimental extract.

B. Use of Luminometers

We recommend using a dedicated luminometer (such as the Tropix NorthStar™ HTS workstation or TR717™ microplate luminometer) to measure the light emission from 96- or 384-well microplates. For most samples, the luminometer can be set to measure for 1-2 min/plate or 0.1-1 sec/well. The linear range of detection will vary according to cell type and on the reporter gene expression level. The number of cells or sample volume used per well should be optimized to prevent a measurement signal that is outside the linear range of the luminometer. Extremely high light signals can saturate the detector, resulting in erroneous measurements. Refer to your luminometer user's manual to determine the upper limit for your specific luminometer. Contact Tropix Technical Support for additional questions or for more information on the NorthStar™ HTS workstation or TR717™ microplate luminometer.

C. Use of Scintillation Counters

A liquid scintillation counter may be used as a substitute for a luminometer, however, sensitivity may be lower (5,6). When using a scintillation counter, it is necessary to turn off the coincident circuit in order to measure bioluminescence directly (single photon counting mode). The manufacturer of the instrument should be contacted to determine how this is done. If it is not possible to turn off the coincident circuit, a linear relationship can be established by taking the square root of the counts per minute measured minus the instrument background.

$$\text{Actual} = (\text{measured} - \text{background})^{1/2}$$

REFERENCES

1. Gallagher, S.R. 1992. Quantitation of GUS activity by fluorometry. *In* GUS Protocols: Using the GUS Gene as a Reporter of Gene Expression, (S.R. Gallagher, ed.), Academic Press, pp. 47-59.

2. Bronstein, I., et al. 1994. Chemiluminescent reporter gene assays: Sensitive detection of the GUS and SEAP gene products. BioTechniques 17:172-178.

3. Hansen, G. and M.D. Chilton. 1996. "Agrolistic" transformation of plant cells: Integration of T-strands generated in planta. Proc. Natl. Acad. Sci. USA 93:14978-14983.

4. Savino, G., et al. 1997. Inhibition of the iron-induced ZmFer1 maize ferritin gene expression by antioxidants and serine/threonine phosphatase inhibitors. J. Biol. Chem. 272:33319-33326.

5. Fulton, R., and B. Van Ness. 1993. Luminescent reporter gene assays for luciferase and β-galactosidase using a liquid scintillation counter. BioTechniques 14:762-763.

6. Nguyen, V.T., et al. 1988. Firefly luciferase luminescence assays using scintillation counters for quantitation in transfected mammalian cells. Anal. Biochem. 171:404-408.

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