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Luciferase Assay Kit

Bioluminescent Reporter Gene Assay
System for the Detection of Firefly Luciferase

Cat. Nos. BC100L, BC300L, BC2500L

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Version 9005-B.1

I. INTRODUCTION

Reporter gene assays are widely used for studying gene regulation and function in cell biology (1,2). Luciferase is a sensitive genetic reporter due to the lack of endogenous activity in cells and tissues. Luciferase assays provide a 1000-fold increase in detection sensitivity compared to the standard assays for chloramphenicol acetyltransferase (CAT) reporter enzyme (3,4). Detection of 1 fg to 20 ng of purified luciferase is possible.

Unlike one-component luciferase assay systems, which exhibit decreased performance after long term storage, the Tropix® Luciferase Assay has been formulated as a two-component system in order to provide consistent results over the life of the kit. Cell lysate is mixed with Substrate A, containing ATP and inorganic salts. Light signal intensity proportional to the amount of enzyme present in an extract is measured immediately after addition of Substrate B, containing luciferin. The enhanced luciferase/luciferin reaction produces a light signal which decays with a half-life of approximately 5 min. The kinetics of light emission enable the use of luminometers with or without automatic injectors and other instrumentation in which light emission measurements can be performed, including scintillation counters (see Appendix C).

II. SYSTEM COMPONENTS

Shelf-life of all Luciferase Assay Kit components is 1 year when stored as recommended below.

	BC100L	BC300L	BC2500L
microplate assays	200	600	5,000
Lysis Solution	70 mL	210 mL	1.75 L
Substrate A	20 mL	3 x 20 mL	5 x 100 mL
Substrate B	20 mL	3 x 20 mL	5 x 100 mL

1. **Lysis Solution:** 100 mM potassium phosphate (pH 7.8), 0.2% Triton X-100. Store at 4°C.

2. **Substrate A:** Lyophilized ATP solution. Reconstitute in 20 mL of H_2O. Store at -20°C before reconstitution; after, store at 4°C for 1 week or store aliquots at -20°C for up to 1 year. Do not re-freeze aliquots.

3. **Substrate B:** Lyophilized luciferin solution. Reconstitute in 20 mL of H_2O. Store at -20°C before reconstitution; after, store at 4°C for 1 week or store aliquots at -20°C for up to 1 year. Do not re-freeze aliquots.

III. LUCIFERASE DETECTION PROTOCOL

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

A. Preparation of Cell Extracts

See Appendix A for preparation of controls.

1. Add DTT (to 1 mM) to the required volume of Lysis Solution (see Note 1).
2. Rinse cell cultures twice with PBS.
3. Add Lysis Solution to cover the cells. Use 250 µL per 60 mm plate.
4. Detach cells from plate with a cell scraper.
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 1 mM) to the required volume of Lysis Solution (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 the Bioluminescent Detection Procedure (Section C), omitting Step 2.

C. Bioluminescent Detection Procedure

Perform all assays in triplicate at room temperature.

1. Equilibrate Substrate A and B to room temperature.

2. Transfer 5-100 µL of extract to luminometer tubes or microplate wells (see Note 2).

3. Add 100 µL of Substrate A.

4. Within 10 min, inject 100 µL of Substrate B. After a 1-2 sec delay, read the signal for 0.1-1 sec (see Note 3).

D. Protocol Notes

1. The Lysis Solution provided may be substituted with alternatives. These should be compared, however, with Lysis Solution in order to ensure optimum assay performance.

2. The amount of extract required may vary depending on the luciferase expression level and the instrumentation used; the amount used should be adjusted to keep the signal within the linear range of the assay. 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. The time between adding Substrate B and start of measurement should be as short as possible, and identical from sample to sample.

APPENDICES

A. Preparation of Controls

Positive Control
Reconstitute lyophilized luciferase (Sigma Cat. No. L-1759) to 1 mg/mL in 0.1 M sodium phosphate (pH 7.0), 0.2% BSA. The stock enzyme should be prepared fresh each time or aliquots can be stored at -70°C and used without repeated freeze/thaw. Generate a standard curve by serially diluting the stock enzyme in 10 µL of Lysis Solution. For the high-end detection limit, use 10-100 ng of enzyme.

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. Alam, J. and J.L. Cook. 1990. Reporter genes: Application to the study of mammalian gene transcription. Anal. Biochem. 188:245-254.

2. Bronstein, I., et al. 1994. Chemiluminescent and bioluminescent reporter gene assays. Anal. Biochem. 219:169-181.

3. Gould, S.J. and S. Subramani. 1988. Firefly luciferase as a tool in molecular and cell biology. Anal. Biochem. 175:5-13.

4. Brasier, A.R., et al. 1989. Optimized use of the firefly luciferase assay as a reporter gene in mammalian cell lines. BioTechniques. 7:1116-1122.

5. Fulton, R., and B. Van Ness. 1993. Luminescent reporter gene assays for luciferase and b-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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