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Dual-Light® System

Chemiluminescent Reporter Gene Assay System
for the Combined Detection of Luciferase and
 β -Galactosidase

Cat. Nos. BD100LP, BD300LP, BD2000LP

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I. INTRODUCTION

Reporter gene assays are widely used for studying gene regulation and function (1,2). The genes encoding luciferase and β -galactosidase are particularly popular due to the availability of highly sensitive, rapid and non-isotopic assays. The Tropix® Dual-Light® reporter gene assay system has been developed for the rapid and sensitive detection of luciferase and β -galactosidase (3-8), enabling experimental and control reporter gene products to be measured in the same cell extract sample. Ultra-high sensitivity and a wide dynamic range are characteristic of the Dual-Light® assay, with detection of 1 fg to 20 ng or 2 fg to 20 ng of purified luciferase or β -galactosidase, respectively.

The Dual-Light® assay incorporates the luminescent luciferin and Tropix Galacton-Plus® substrates for the detection of luciferase and β -galactosidase, respectively. Cell lysate is mixed with Buffer A for the luciferase reaction; the luciferase signal is measured immediately after the addition of Buffer B, which contains the substrates. The enhanced luciferase reaction produces a light signal which decays with a half-life of approximately 1 min. Light signal from the β -galactosidase reaction is initially negligible due to the low reaction pH (7.8) and

absence of enhancer. After a 30-60 min incubation, light signal from the accumulated product of the β -galactosidase/Galacton-Plus[®] substrate reaction is initiated by addition of light emission Accelerator-II which raises the pH and provides Tropix Sapphire-II[™] luminescence enhancer to increase light intensity. Light emission from the β -galactosidase reaction exhibits glow kinetics with a half-life of 180 min. Residual light from the luciferase reaction is minimal, due to rapid kinetic decay and the quenching effect of Accelerator-II. Generally, only very high amounts of luciferase (ng levels of enzyme) interfere with detection of β -galactosidase. A longer delay after addition of Accelerator-II prior to measurement will result in decreased residual luciferase signal when extremely high levels are present. However, it is important to maintain consistent timing of addition of Buffer B and measurement of the β -galactosidase signal after adding Accelerator-II.

It is very important to stay within the linear range of the assay for both enzymes. High intensity signals can saturate a photo-multiplier tube resulting in artificially low signals. In addition, low signals that approach background may also be outside the linear range. In these cases, the amount of cell extract used in the assay should be adjusted.

The Dual-Light[®] system is suitable for use with luminometers with automatic injectors and other instrumentation in which light emission measurements can be acquired quickly. If only a single injector is available, it should be rinsed thoroughly between injection of Buffer B and Accelerator-II. Manual injection may be performed if luminescence intensities are measured at the same interval after adding Accelerator. The volume of cell extract should be 2-10 μ L. The lysis solution included may be substituted with alternative solutions. However, reducing agents interfere with the Galacton-Plus[®] substrate, causing high background and rapid signal decay. Alternative lysis solutions should be carefully evaluated to ensure optimum performance.

High levels of endogenous β -galactosidase activity in samples may interfere with measurement of reporter. Endogenous enzyme activity is reduced at the reaction pH (9), however, it is important to assay the level of endogenous enzyme with non-transfected extracts. Heat inactivation to reduce endogenous activity should not be performed prior to a Dual-Light[®] assay due to a detrimental effect on luciferase. If high endogenous activity necessitates heat inactivation, assays for luciferase and β -galactosidase should be performed individually.

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II. SYSTEM COMPONENTS

Shelf-life for all Dual-Light[®] kit components is 1 yr when stored as indicated below.

	BD100LP	BD300LP	BD2000LP
microplate assays	200	600	4,000
Lysis Solution	70 mL	210 mL	1.4 L
Buffer A	5 mL	3 x 5 mL	20 x 5 mL
Galacton-Plus [®] Substrate	200 μ L	600 μ L	4 mL
Buffer B	22 mL	3 x 22 mL	20 x 22 mL
Accelerator-II	25 mL	75 mL	500 mL

NOTE: Dithiothreitol (DTT, not included) may be added fresh to Lysis Solution prior to use to a final concentration of 0.5 mM to preserve luciferase activity. However, higher concentrations of reducing agents may increase the background of the β -galactosidase assay and will decrease the half-life of light emission of Galacton-Plus[®] substrate. If extended light emission is critical, reducing agents should be omitted. If a buffer containing excess DTT has been used, the addition of hydrogen peroxide to the Accelerator to a final concentration of 10 mM (add 1 μ L of 30% H₂O₂ per 1 mL of Accelerator) will prevent rapid decay of signal half-life.

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- Lysis Solution:** 100 mM potassium phosphate pH 7.8, 0.2% Triton X-100. Store at 4°C.
- Buffer A:** Lyophilized powder. Reconstitute in 5 mL of water. Store at -20°C before reconstitution. After reconstitution, store at 4°C for 1 week or aliquot and store at -20°C.
- Galacton-Plus[®] Substrate:** 100X concentrate. Store at 4°C.
- Buffer B:** Lyophilized luciferin. Reconstitute in 22 mL of water. Store at -20°C before reconstitution. After reconstitution, store at 4°C for 1 week or aliquot and store at -20°C.
- Light Emission Accelerator-II:** Ready-To-Use solution containing Sapphire-II[™] enhancer. Store at 4°C.

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III. LUCIFERASE AND β -GALACTOSIDASE DETECTION PROTOCOL

Please read the entire Protocol and Notes sections before proceeding.

A. Preparation of Extracts From Cultured Cells

See Note 1 on optimization of transfections.

1. Add DTT (to 0.5 mM) to the required volume of Lysis Solution (if desired, see Note 2).
2. Rinse cell cultures twice with PBS.
3. Add Lysis Solution to cover the cells. Use 250 μ L 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 .

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C. Chemiluminescent Detection Protocol

Perform all assays in triplicate at room temperature.

1. Equilibrate Buffer A and B to room temperature.
2. Dilute Galacton-Plus[®] substrate 1:100 in Buffer B. Prepare only enough for immediate use (100 μ L/tube or well).
3. Transfer 2-10 μ L of extracts to luminometer tubes or microplate wells (see Note 3).
4. Add 25 μ L of Buffer A to the extract samples.
5. Within 10 min, inject 100 μ L of Buffer B. After a 1-2 sec delay, read the luciferase signal for 0.1-1 sec/well (see Note 4).
6. Incubate for 30-60 min at room temperature.
7. Inject 100 μ L of Accelerator-II. After a 1-2 sec delay, read the β -galactosidase signal for 0.1-1 sec/well (see Note 4).

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B. Direct Lysis Protocol for Microplate Cultures

This procedure is designed for adherent cells growing in 96-well tissue culture-treated luminometer plates. Perform assays in triplicate at room temperature. Heat inactivation of endogenous galactosidase activity is not effective with this protocol.

1. Add DTT (to 0.5 mM) to the required volume of Lysis Solution (if desired, see Note 2).
2. Rinse cell cultures once with PBS.
3. Add 10 μ L of Lysis Solution to each well and incubate for 10 min.
4. Continue with the Chemiluminescent Detection Protocol (Section C), omitting Step 3.

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D. Protocol Notes

1. With Dual-Light[®] assays, the same aliquot of extract is assayed for each enzyme. The ratio of the vectors used in transfections should be optimized to ensure that signal intensities are within the linear detection range of the assay (1 fg to 20 ng of enzyme for luciferase and 2 fg to 20 ng for β -galactosidase) and the luminometer being used.
2. DTT may help to preserve luciferase activity, but it may have adverse effects on the background and kinetics of the β -galactosidase assay.
3. The amount of extract used should be optimized to keep signals in the linear range of the assay. Use Lysis Solution to bring the total volume to 10 μ L for every sample.
4. If using an instrument without injectors, other delay and read times may be used. However, be sure that the same timing is used when measuring the β -galactosidase signal after addition of Accelerator-II; Accelerator-II must be added in the same consistent time frame as Buffer B (Galacton-Plus[®]) addition.

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APPENDICES

A. Preparation of Controls

Positive Control

β-Galactosidase: Reconstitute lyophilized β-galactosidase (Sigma, G-5635) 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. 2-20 ng of enzyme should be used as an upper detection limit.

Luciferase: 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 -80°C and used without repeated freeze/thaw. Generate a standard curve by serially diluting in Lysis Solution containing 0.1% BSA. 2-20 ng of enzyme should be used 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 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 0.1 sec per 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 TR717™ microplate luminometer.

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. Bourcier, T., et al. 1997. The nuclear factor κ-B signaling pathway participates in dysregulation of vascular smooth muscle cells *in vitro* and in human atherosclerosis. *J. Biol. Chem.* 272:15817-15824.
4. Bronstein, I., et al. 1996. Chemiluminescence: Sensitive detection technology for reporter gene assays. *Clin. Chem.* 42:1542-1546.
5. Bronstein, I., et al. 1997. Combined luminescent assays for multiple enzymes, p. 451-457. In *Bioluminescence and Chemiluminescence: Molecular Reporting with Photons* (Hastings, J.W., Kricka, L.J. and Stanley, P.E., eds.), John Wiley, Chichester, England.
6. Hollenberg, A.N., et al. 1997. Functional antagonism between CCAAT/enhancer binding protein-α and peroxisome proliferator-activated receptor-γ on the leptin promoter. *J. Biol. Chem.* 272:5283-5290.
7. Martin, C.S., et al. 1996. Dual luminescence-based reporter gene assay for luciferase and β-galactosidase. *BioTechniques* 21:520-524.
8. Moessler, H., et al. 1996. The SM22 promoter directs tissue-specific expression in arterial but not in venous or visceral smooth muscle cells in transgenic mice. *Development* 122:2415-2425.
9. Jain, V. and I. Magrath. 1991. A chemiluminescent assay for quantitation of β-galactosidase in the femtogram range: Application to quantitation of β-galactosidase in *lacZ*-transfected cells. *Anal. Biochem.* 199:119-124.

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