

Phone: (800) 542-2369 or
(781) 271-0045
FAX: (781) 275-8581

e-mail: tropix@appliedbiosystems.com
<http://www.appliedbiosystems.com/tropix>

Applied Biosystems, Dual-Light, Galacton, Galacton-Plus, Galacton-Star and Tropix are registered trademarks, and Applera Galacton-Star, NorthStar, Sapphire-II and TR717 are trademarks of Applera Corporation or its subsidiaries in the US and certain other countries. For Research Use Only. Not for use in diagnostic procedures. Information is subject to change without notice.

© Copyright 2000 Applied Biosystems. Printed in USA.
Version 9017-C.1



Rear
Cover

Galacto-Star™ System

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

Cat. Nos. BM100S, BM300S, BM2500S
BY100S and BY300S, BY2500S

<u>Contents</u>	<u>PAGE</u>
I. INTRODUCTION	1
II. SYSTEM COMPONENTS	4
III. β -GALACTOSIDASE DETECTION PROTOCOL	
A. Preparation of Mammalian Cell Extracts	5
B. Preparation of Yeast Cell Extracts	6
C. Direct Lysis Protocol for Microplate Cultures	7
D. Detection with Tube Luminometers	8
E. Detection with Microplate Luminometers	9
F. Protocol Notes	10
APPENDICES	
A. Preparation of Controls	11
B. Inactivation of Endogenous β -Galactosidase	12
C. Use of Luminometers	13
D. Use of Scintillation Counters	14
REFERENCES	15

Front
Cover

I. INTRODUCTION

The Tropix® Galacto-Star™ chemiluminescent reporter gene assay system is designed for rapid and sensitive detection of β -galactosidase activity in cell lysates. The assay incorporates Tropix Galacton-Star® substrate for β -galactosidase with Sapphire-II™ luminescence enhancer to produce glow light emission kinetics. The Galacto-Star™ system has a wide dynamic range, enabling detection from 2 fg to 20 ng of purified β -galactosidase (1). The system is ideally suited for use with mammalian or yeast cells, including two-hybrid (2) and one-hybrid (3) interaction trap assays.

The Galacto-Star™ assay provides a simplified method for detecting β -galactosidase. Cell lysate is incubated with Reaction Buffer containing Galacton-Star® substrate and Sapphire-II™ enhancer until maximum light emission is reached (typically 30-90 min, depending upon assay temperature (Fig. 1); after peak, light emission remains constant for nearly 1 hour. Light output is measured in a luminometer or scintillation counter without the need for reagent injection. A direct lysis protocol for measurement of β -galactosidase activity in microplate cell cultures is also provided.

The bacterial β -galactosidase gene is widely

used as a reporter for the study of gene regulation, and more recently in systems for identification of protein:protein interactions. Tropix 1,2-dioxetane substrates for β -galactosidase, including Galacton[®], Galacton-Plus[®] and Galacton-Star[®] substrates, provide highly sensitive enzyme detection (1,4-6), have been used in reporter assays in both cell and tissue extracts (7,8), and have been adapted to a combined assay for luciferase and β -galactosidase (Dual-Light[®] system, [9,10]).

Galacton[®] substrates have been utilized with yeast extracts (11,12), including the one- or the two-hybrid system (13,14), or for reporter assays in protozoa (15), bacteria (16), or frog embryo extracts (17). A novel β -galactosidase complementation system in mammalian cells to report protein:protein interactions has been performed with Galacton-Plus[®] substrate (18,19). In addition, peptide complementation has been used in an assay for myoblast cell fusion (18,20). Galacton[®] substrate has been used in a cytotoxicity assay based on release of β -galactosidase enzyme from transfected cells (21).

Chemiluminescent reporter assays may be conducted in cells or tissues that have endogenous β -galactosidase. Reduction of such activity may be

2

II. SYSTEM COMPONENTS

Shelf-life for all Galacton-Star[™] kit components is 1 yr at 4°C.

	B'X'100S	B'X'300S	B'X'2500S
microplate assays	600	1,800	15,000
Lysis Solution ('BM' kits)	70 mL	210 mL	1.75 L
OR 5X Z Buffer ('BY' kits)	80 mL	240 mL	2 L
Galacton-Star [®] Substrate	1.2 mL	3.6 ml	30 mL
Reaction Buffer Diluent	60 mL	180 ml	1.5 L

- Lysis Solution:** 100 mM potassium phosphate (pH 7.8), 0.2% Triton X-100 (for mammalian cells, in BM100S, BM300S and BM2500S kits).

OR

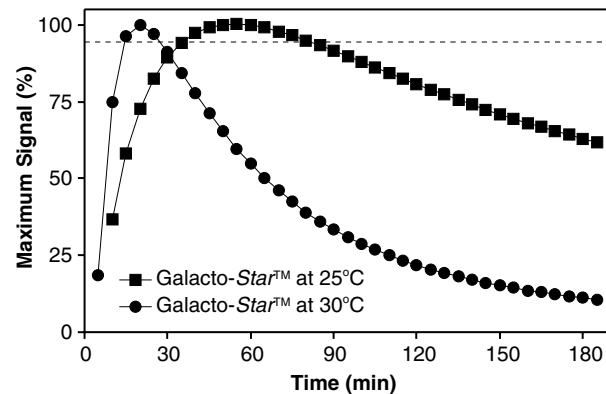
5X Z Buffer: 0.5 M sodium-phosphate (pH 7.1), 50 mM KCl, 5 mM MgSO₄ (for yeast cells, in BY100S, BY300S and BY2500S kits).

- Galacton-Star[®] Substrate:** 50X concentrate.
- Reaction Buffer Diluent:** 100 mM sodium phosphate (pH 7.5), 1 mM MgCl₂, 5% Sapphire-II[™] enhancer.

4

achieved using heat inactivation (22). Analysis of tissue extracts may require the use of protease inhibitors (8).

Figure 1



Galacton-Star[™] assays were performed with purified β -galactosidase (500 pg, diluted in 16 μ l of PBS). Repeated measurements were made in the Tropix TR717[™] microplate luminometer at either room temperature (25°C, squares) or 30°C (circles). The data is presented as the percentage of the maximum signal. The dotted line indicates 95% of maximum signal.

3

III. β -GALACTOSIDASE DETECTION PROTOCOL

Please read the entire Protocol and Notes sections before proceeding.

A. Preparation of Mammalian Cell Extracts

Non-adherent cells should be pelleted and rinsed. Sufficient Lysis Solution should be added to cover the pellet, then the cells should be resuspended by pipetting. Continue at step 5 below.

- Add DTT (to 0.5 mM) to the required volume of Lysis Solution (if desired, see Note 1).
- Rinse cell cultures twice with PBS.
- Add Lysis Solution to cover the cells. Use 250 μ L per 60 mm plate.
- Detach cells from plate with a cell scraper.
- Transfer the cell lysate to a microfuge tube and centrifuge for 2 min to pellet debris.
- Transfer extracts (supernatant) to a fresh tube. Use immediately or store at -70°C.

5

B. Preparation of Yeast Cell Extracts

1. Dilute 5X Z Buffer 1:5 in H₂O. Add DTT to 0.5 mM (if desired, see Note 1).
2. Transfer 1.5 mL of yeast culture (OD₆₀₀ = 0.5) to a microfuge tube. Centrifuge at 12,000xg for 30 sec. If OD₆₀₀ is < 0.4, use > 1.5 mL.
3. Remove supernatant and resuspend cell pellet in 1.5 mL of 1X Z Buffer.
4. Centrifuge at 12,000xg for 30 sec.
5. Remove supernatant and resuspend pellet in 300 µL of 1X Z Buffer.
6. Transfer 100 µL to a fresh tube. Store the remainder at 4°C.
7. Perform 2 freeze/thaw cycles. Freeze in liquid N₂ for 60 sec. Thaw cells at 37°C for 60 sec.
8. Centrifuge at 12,000xg for 5 min at 4°C.
9. Transfer supernatant to a fresh tube. Use immediately or store at -70°C.

6

C. 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 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 procedure for Detection with Microplate Luminometers (Section E), omitting Step 2.

7

D. Detection with Tube Luminometers

Perform assays in triplicate at room temperature.

1. Dilute Galacton-*Star*[®] substrate 1:50 with Reaction Buffer Diluent to make Reaction Buffer. Prepare only enough for immediate use (300 µL/tube). Equilibrate to room temperature.
2. Transfer 2-20 µL of extract to luminometer tubes (see Note 2).
3. Add 300 µL of Reaction Buffer, mix, and incubate for 30-90 min until light emission is maximal (see Figure 1 and Note 3).
4. Measure the signal in a luminometer for 0.1-1 sec/tube.

8

E. Detection with Microplate Luminometers

Perform assays in triplicate at room temperature.

1. Dilute Galacton-*Star*[®] substrate 1:50 with Reaction Buffer Diluent to make Reaction Buffer. Prepare only enough for immediate use (100 µL/well). Equilibrate to room temperature.
2. Transfer 2-10 µL of extract to microplate wells (see Note 2).
3. Add 100 µL of Reaction Buffer, mix, and incubate for 30-90 min until light emission is maximal (see Figure 1 and Note 3).
4. Measure light signal in a luminometer for 0.1-1 sec/well.

9

APPENDICES

A. Preparation of Controls

Positive Control

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.

Negative Control

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

F. Protocol Notes

1. Dithiothreitol (DTT) may be added to Lysis Solution (to 0.5 mM) to stabilize β -galactosidase activity. However, DTT may increase assay background, and high concentrations will decrease light emission half-life. If low background and extended half-life is critical, DTT should be omitted.
2. The amount of extract required may vary depending on the β -galactosidase expression level and the instrumentation used. Use 2-5 or 10-20 μ L 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 or 1X Z Buffer may be added to equalize sample volumes.
3. Measurements can be performed after 20-30 min if the time between Reaction Buffer addition and light measurement is the same for each tube.

C. 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 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.

B. Inactivation of Endogenous β -Galactosidase

Some cell lines exhibit endogenous β -galactosidase activity. This may lead to high backgrounds which will decrease the sensitivity of the assay. A procedure for heat inactivation of endogenous β -galactosidase activity has been described (22). A modified version of this protocol has been described for use with tissue in which protease inhibitors (PMSF and leupeptin) are used in conjunction with the heat inactivation procedure (8). These procedures should be performed prior to the Detection Protocol.

Inactivation of Endogenous β -Galactosidase

1. Heat the extract for 50-60 min at 48°C.
2. Proceed with Detection (Section IIIC or IIID).

NOTE: PMSF (to 0.2 mM) and leupeptin (to 5 μ g/mL) may be added to Lysis Solution just before use if protease activity may be present.

NOTE: AEBSF (a water-soluble analog of PMSF; Sigma A-8456) may replace PMSF (Sigma P-7626).

D. Use of Scintillation Counters

A liquid scintillation counter may be used as a substitute for a luminometer, however, sensitivity may be lower (23,24). When using a scintillation counter to measure chemiluminescence, it is necessary to turn off the coincident circuit (single photon counting mode). The instrument manufacturer 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. Martin, C.S., et al. 1997. Continuous sensitive detection of β -galactosidase with a novel chemiluminescent 1,2-dioxetane, p. 525-528. In *Bioluminescence and Chemiluminescence: Molecular Reporting with Photons* (Hastings, J.W., Kricka, L.J. and Stanley, P.E., eds.), John Wiley, Chichester, England.
2. Fields, S. and O. Song. 1989. A novel genetic system to detect protein-protein interactions. *Nature* 340:245-246.
3. Li, J. and I. Herskowitz. 1993. Isolation of ORC6, a component of the yeast origin recognition complex by a one-hybrid system. *Science* 262:1870-1874.
4. Bronstein, I., et al. 1994. Chemiluminescent reporter gene assays for β -galactosidase, β -glucuronidase and secreted alkaline phosphatase, p. 20-23. In *Bioluminescence and Chemiluminescence: Fundamentals and Applied Aspects* (Campbell, A.K., Kricka, L.J., and Stanley, P.E., eds.), John Wiley, Chichester, England.
5. Bronstein, I., et al. 1996. Chemiluminescence: Sensitive detection technology for reporter gene assays. *Clin. Chem.* 42:1542-1546.
6. Jain, V.K. and I.T. 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.
7. O'Connor, K.L. and L.A. Culp. 1994. Topological and quantitative analyses of early events in tumor formation using histochemically-tagged transformed 3T3 cells. *Oncology Reports* 1:869-876.
8. Shaper, N., et al. 1994. Male germ cell expression of murine β 4-galactosyltransferase. A 796-base pair genomic region, containing two cAMP-responsive element (CRE)-like elements, mediates male germ cell-specific expression in transgenic mice. *J. Biol. Chem.* 269: 25165-25171.
9. 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.
10. Martin, C.S., et al. 1996. Dual luminescence-based reporter gene assay for luciferase and β -galactosidase. *BioTechniques* 21:520-524.
11. Remacle, J.E., G. et al. 1997. Three classes of mammalian transcription activation domain stimulate transcription in *Schizosaccharomyces pombe*. *EMBO J.* 16:5722-5729.
12. Stoldt, V.R., et al. 1997. Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes of fungi. *EMBO J.* 16:1982-1991.
13. Bourne, Y., et al. 1996. Crystal structure and mutational analysis of the human CDK2 kinase complex with cell-cycle regulatory protein CksHs1. *Cell* 84:863-874.
14. Wolf, S.S., et al. 1996. Construction of a reporter plasmid that allows expression libraries to be exploited for the one-hybrid assay system. *BioTechniques* 20:568-573.
15. Beetham, J.K., et al. 1997. Glycoprotein 46 mRNA abundance is post-transcriptionally regulated during development of *Leishmania chagasi* promastigotes to an infectious form. *J. Biol. Chem.* 272:17360-17366.
16. Ding, L. and M. Navre. 1996. Chemiluminescent assay for β -galactosidase activity in *E. coli* lysates. *Luminescence Topics* 5(1):2.
17. Gove, C., et al. 1997. Over-expression of GATA-6 in *Xenopus* embryos blocks differentiation of heart precursors. *EMBO J.* 16:355-368.
18. Mohler, W.A. and H.M. Blau. 1996. Gene expression and cell fusion analyzed by *lacZ* complementation in mammalian cells. *Proc. Natl. Acad. Sci. USA* 93:12423-12427.
19. Rossi, F., et al. 1997. Monitoring protein-protein interactions in intact eukaryotic cells by β -galactosidase complementation. *Proc. Natl. Acad. Sci. USA* 94:8405-8410.
20. Charlton, C.A., et al. 1997. Fusion competence of myoblasts rendered genetically null for N-cadherin in culture. *J. Cell Biol.* 138:331-336.
21. Schafer, H., et al. 1997. A highly sensitive cytotoxicity assay based on the release of reporter enzymes, from stably transfected cell lines. *J. Immunol. Methods* 204:89-98.
22. Young, D.C., et al. 1993. Selective inactivation of eukaryotic β -galactosidase in assays for inhibitors of HIV-1 TAT using bacterial β -galactosidase as a reporter enzyme. *Anal. Biochem.* 215:24-30.
23. 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.
24. Nguyen, V.T., et al. 1988. Firefly luciferase luminescence assays using scintillation counters for quantitation in transfected mammalian cells. *Anal. Biochem.* 171:404-408.

WARRANTY

Tropix, a wholly owned subsidiary of Applera Corporation (formerly PE Corporation) ("Tropix"), warrants its products, to only the original purchaser and to no third party, against defects in materials and workmanship under normal use and application. Tropix' sole obligation and total liability under this warranty shall be to replace defective products. All products are supplied For Research Use Only as defined herein and are Not For Resale. Commercialization of products using these components requires an express license under applicable patents and intellectual property from Tropix that is not included herein. Our preparations are intended exclusively for in vitro use only. They are not for diagnostic or therapeutic use in humans or animals. Without limiting or otherwise affecting the limitations on warranty and liability stated herein: (1) those preparations with known toxicity are sent with an information sheet which describes, to our knowledge, the potential dangers in handling, (2) the absence of a toxicity warning with one of our products does not preclude a possible health hazard, (3) with all of our products, due care should be exercised to prevent human contact and ingestion, and (4) preparations should be handled by trained personnel only. This warranty is in lieu of all other warranties, express or implied and, without limitation, Tropix EXPRESSLY DISCLAIMS THE WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE. IN NO CASE OR EVENT SHALL TROPIX BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES, OF WHATEVER NATURE, EVEN IF TROPIX HAS BEEN ADVISED OF THE POSSIBILITY THEREOF.

Notes

Notes