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Gal-Screen® System

Chemiluminescent Reporter Gene Assay System

for the Detection of β-Galactosidase in Mammalian or Yeast Cell Cultures

P/N T1027, T1028, T1029, T1030, T1031, T1032

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Part Number T9020 Revision D **Revision Date: October 2008**

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Literature Citation: When describing a procedure for publication using this product, please refer to it as the Gal-Screen® System.

PREFACE

Safety Information

Note: For general safety information, see this Preface and Appendix C, “Safety” on page 5. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the “Safety” Appendix for the complete alert on the chemical or instrument.

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at point in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

MSDSs

The MSDSs for any chemicals supplied by Applied Biosystems are available to you free 24 hours a day. For instructions on obtaining MSDSs, see MSDSs on page 6.

IMPORTANT! For the MSDSs of chemicals not distributed by Applied Biosystems contact the chemical manufacturer.

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- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
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- Submit a question directly to Technical Support.
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- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

I. INTRODUCTION

The Gal-Screen chemiluminescent reporter gene assay system is designed for the rapid, simple and sensitive detection of β-galactosidase reporter enzyme directly in microwell cultures of mammalian or yeast cells. The Gal-Screen reporter assay system incorporates the Galacton-*Star*® substrate with a luminescence enhancer to generate glow light emission kinetics. A single reagent, which provides cell lysis components and luminescent substrate and enhancer, is added directly to cells in culture medium. Light emission typically reaches maximum intensity within 60-90 minutes and remains constant for 45-90 minutes or longer (depending upon assay temperature, see Fig. 1). Light signal is measured in a luminometer, without the need for automatic injection. The simple assay format and glow light emission achieved with the Gal-Screen reagents provide an ideal assay system for automated high throughput screening applications, enabling simple processing and measurement of multiple microplates. The dynamic range of the assay spans five orders of magnitude, from picogram to nanogram levels, enabling detection of a wide range of reporter enzyme concentration in cells.

Applications

The bacterial β-galactosidase gene is widely used as a reporter enzyme for the study of gene regulation, for identification of protein-protein interactions and in assays for cell fusion. Chemiluminescent 1,2-dioxetane substrates for β-galactosidase, including Galacton® (product discontinued), Galacton-Plus® and Galacton-*Star* ® substrates, provide highly sensitive enzyme detection (1-4) and have been utilized extensively in reporter assays in both mammalian cell culture extracts and tissue extracts, and in a combined assay for luciferase and β-galactosidase activities (5,6; Dual-Light® assay system, P/N T1000). The Gal-Screen assay system is used widely for traditional reporter gene assays in transiently and stably-transfected mammalian cells (7,8), including assays for studying viral infectivity and function (9-12). It is widely used for reporter gene assays in yeast cells (13-15), including quantitative yeast two hybrid (17-19) and one hybrid analysis (20). In addition, it has been used for reporter gene assays in fish cells (21) and bacterial cells (22). Gal-Screen assays provide highly sensitive detection for β-galactosidase complementation assays used for intracellular monitoring of protein-protein interactions (23), protein translocation (24), cell fusion (25), and receptor dimerization/activation (26,27), including for high throughput compound screening for receptor activation (28).

The Gal-Screen reporter gene assay system provides a chemiluminescent assay format that is sensitive, simple to use, and designed specifically for optimal performance in automated, high throughput screening applications. The Gal-Screen assay protocol is adaptable for use in multiple microplate formats, with either mammalian or yeast cells.

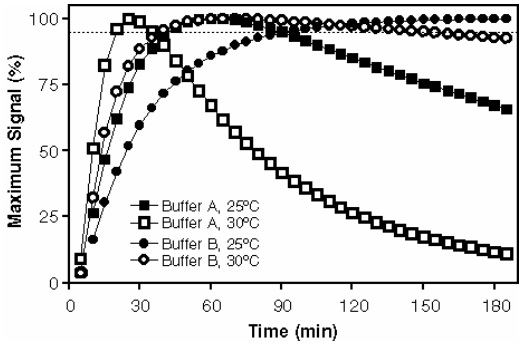

Figure 1. Gal-Screen assays were performed with purified β-galactosidase (500 pg) with either Buffer A or Buffer B. Repeated measurements were made in the Applied Biosystems TR717™ microplate luminometer at either room temperature (25°C) or 30°C. Data is presented as percentage of maximum signal, and dotted line indicates 95% of maximum signal.

II. SYSTEM COMPONENTS

Shelf-life for all Gal-Screen® assay components is 1 yr at 4°C.

	T1029, T1032	T1027, T1030	T1028, T1031
Microplate assays per kit	200	1000	10,000
Gal-Screen® substrate	0.8 mL	4 mL	40 mL
Gal-Screen® Buffer A or B	19.2 mL	96 mL	960 mL

1. **Gal-Screen® Substrate:** Galacton-*Star*® formulation which is diluted 1:25 in Gal-Screen Buffer A or B prior to use.

2. **Gal-Screen® Buffer A or Buffer B:** contain luminescence enhancer and lysis reagents.

   T1029, T1027, T1028: contain Gal-Screen® Buffer A, for use with mammalian cells

   T1032, T1030, T1031: contain Gal-Screen® Buffer B, for use with yeast or mammalian cells

III. β-GALACTOSIDASE DETECTION PROTOCOL

Please read the entire Protocol and Notes sections before proceeding. All assays should be performed in duplicate or triplicate. See Appendix A for preparation of positive and negative controls.

1. Dilute Gal-Screen® substrate 1:25 with Gal-Screen® Buffer A or B (ie., mix 40 µl Gal-Screen Substrate + 960 µl Gal-Screen Buffer A or B, 100 µl/well required, see Note 1) to make Reaction Buffer A or B. Equilibrate Reaction Buffer A or B to room temperature before use. Gal-Screen substrate should be diluted fresh each time.

2. Add 100 µl/well of Reaction Buffer A or B to a 96-well microplate containing 100 µl/well of cells in culture medium (see Notes 2-6). Incubate at 26-28°C for approximately 60-90 minutes or until constant light emission is reached (see Note 7).

3. Place microplate in a luminometer and measure for 0.1-1 sec/well.

IV. PROTOCOL NOTES

1. Gal-Screen Buffer A is optimized for mammalian cell cultures, and has been used with transfected adherent and non-adherent cell lines, including NIH/3T3, CHO-K1 and K562. Gal-Screen Buffer B was optimized for yeast cultures, and has subsequently been shown to work with mammalian cell cultures also. The alternative buffer formulations result in differing light emission kinetics. For mammalian cells, Reaction Buffer A provides faster kinetics, which may be advantageous at lower incubation temperatures. The choice of Gal-Screen reagents will depend on the particular assay requirements and desired kinetic performance.

2. The use of white, tissue culture-treated microplates is recommended for optimal assay sensitivity. Clear-bottom white plates can be utilized to allow microscopic examination of cultures. White backing sheets may be applied to the plate bottom prior to signal measurement if desired. The absolute signal will be higher (approximately 2-fold), since the white backing reflects light toward the photomultiplier tube detector and eliminates light absorption by the black plate platform, but relative signal levels are unaffected. Black plates can be used but are not recommended, since they will cause reduced light yield due to absorbance of the luminescent signal.

3. 384-Well plates can be used with the Gal-Screen assay. Culture volume and assay reagent volume should be reduced to 25 µl (or as desired, maintaining a 1:1 ratio of culture volume:reagent volume without overfilling the wells).

4. For mammalian cell cultures, a typical cell density is 10,000-50,000 cells/well in 100 µl for 96-well plates, or 1000-10,000 cells/well in 25 µl for 384-well plates. For yeast cell cultures, a typical cell density is 10,000-75,000 cells/well in 100 µl for 96-well plates, or 5,000-40,000 cells/well in 25 µl for 384-well plates.

5. Cell culture medium (mammalian cells) containing phenol red indicator can be used without affecting assay sensitivity. The inclusion of phenol red can result in a reduction of signal, due to absorbance of some of the emitted light, but the signal/noise is not affected. The type of culture medium and presence/absence of serum may contribute to some slight variability in light signal and assay kinetics. It is recommended to initially perform a kinetic analysis of the particular cell line/culture medium system to determine optimum signal measurement time. It is not necessary to measure light signal at the time of peak light emission, as long as all samples are measured in a consistent time frame.

6. Chemiluminescent β-galactosidase reporter assays may be conducted in mammalian cells that have endogenous β-galactosidase activity. The reaction is performed at a pH which is more favorable for bacterial enzyme activity, which has a higher pH optimum than the endogenous mammalian enzyme. It is important to assay the level of endogenous enzyme in non-transfected cells to determine the assay background signal.

7. Assays are ideally performed at 26-28°C. If the incubation temperature is significantly different, the kinetics of light emission will vary, since the rate of an enzyme reaction is dependent on temperature (see Figure 1).

V. APPENDICES

A. Preparation of Controls

Positive Control

β-Galactosidase: Prepare stock enzyme by reconstituting 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 the stock enzyme in cell culture medium. For the high end of the dilution curve, use 2-20 ng of enzyme. Purified enzyme provides a positive control for the assay reagents, as well as a means to determine the range of detection of the luminometer instrumentation, if desired. The purified enzyme standard curve is not intended (or accurate) for absolute quantitation of reporter enzyme concentrations, as the specific activity of the purified enzyme preparation and the reporter enzyme may differ significantly. Additional positive controls can include use of control β-galactosidase constructs that provide constitutive expression of reporter enzyme as a positive control for cell transfection.

Negative Control
Assay a volume of mock-transfected extract equivalent to that of experimental extract to determine endogenous cellular background. In experiments involving induction of reporter expression, uninduced cells should be assayed as a negative control for total assay background.

B. Use of Luminometers

We recommend using a single-mode luminometer or a multi-mode detection instrument set for luminescence measurement to measure light emission from 96- or 384-well microplates. The linear range of detection will vary according to cell type and on the reporter enzyme expression level. The number of cells 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 (very unlikely for experimental samples), resulting in erroneous measurements. Refer to your luminometer user's manual to determine the upper limit for your specific luminometer. Contact Applied Biosystems Technical Support group for additional questions.

C. Safety

GENERAL CHEMICAL SAFETY

Chemical hazard warning

⚠ **WARNING! CHEMICAL HAZARD.** Before handling any chemicals, refer to the Material Safety Data Sheet (MSDS) provided by the manufacturer, and observe all relevant precautions.

⚠ **WARNING! CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

⚠ **WARNING! CHEMICAL HAZARD.** Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

⚠ **WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page 6.)

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

MSDSs

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to new customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to www.appliedbiosystems.com, click **Support**, then select **MSDS**.

2. In the Keyword Search field, enter the chemical name, product name, MSDS part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click Search.

3. Find the document of interest, right-click the document title, then select any of the following:
    - **Open** – To view the document
    - **Print Target** – To print the document
    - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

CHEMICAL WASTE SAFETY

Chemical waste hazards

⚠ **CAUTION! HAZARDOUS WASTE.** Refer to Material Safety Data Sheets and local regulations for handling and disposal.

⚠ **WARNING! CHEMICAL WASTE HAZARD.** Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

⚠ **WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

  IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

BIOLOGICAL HAZARD SAFETY

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories (stock no. 017-040-00547-4; bmbl.od.nih.gov)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

IMPORTANT! Additional information about biohazard guidelines is available at: www.cdc.gov

CHEMICAL ALERTS

For the definitions of the alert words **IMPORTANT**, **CAUTION**, **WARNING**, and **DANGER**, see "Safety alert words" on page 1.

General alerts for all chemicals

EXAMPLE: Avoid contact with (skin, eyes, and/or clothing). Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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For complete, updated reference list, please see http://www.appliedbiosystems.com (Product & Service Literature).