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Dual-Light® System

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

P/N T1003, T1004, T1005

Contents **Page**

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For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

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

PREFACE

Safety Information

Note: For general safety information, see this Preface and Appendix C, “Safety” on page 6. 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 7.

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

How to Obtain Support

For the latest services and support information for all locations, go to:

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At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents.
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I. INTRODUCTION

Reporter gene assays are widely used for studying gene regulation and function. The genes encoding firefly luciferase and β-galactosidase are particularly popular due to the availability of highly sensitive, rapid detection assays. The Tropix® Dual-Light® reporter gene assay system was developed for the rapid and sensitive sequential detection of firefly luciferase and β-galactosidase, enabling experimental and control reporter gene enzymes to be measured in the same cell extract sample (1,2). Ultra-high sensitivity and a wide dynamic range are attained with the Dual-Light assay, with detection of 1 fg to 20 ng or 10 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 injection of Buffer B, which contains luciferin and Galacton-Plus substrates. The enhanced luciferase reaction produces a light signal which decays with a half-life of approximately 1 minute. Light signal from the β-galactosidase reaction is negligible due to lack of enzyme turnover time, low pH (7.8) and absence of enhancer. After a 30-60 minute incubation, light signal from the accumulated product of the β-galactosidase/Galacton-Plus reaction is initiated by addition of Accelerator-II which raises the pH and provides 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 signal decay and quenching by Accelerator-II. Generally, only very high luciferase concentrations (ng levels of enzyme) interfere with detection of β-galactosidase. A longer delay after the 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 important to stay within the linear range of the assay for both enzymes. High intensity signals can potentially saturate a photo-multiplier tube resulting in artificially low signals (this is unlikely with typical experimental samples). In addition, low signals that approach background levels may be outside the linear range. The amount of cell extract used should be adjusted to ensure the assay is within the linear range.

The Dual-Light system is suitable for use with luminometers with automatic injectors. If only a single injector is available, it should be rinsed thoroughly between injection of Buffer B and Accelerator-II. Manual addition of Accelerator-II may be performed if luminescence intensities are measured at the same interval after adding Accelerator-II. The lysis solution included may be substituted with alternative lysis solutions, as long as all samples are prepared with the same lysis solution. 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 desired assay performance.

High levels of endogenous mammalian β-galactosidase activity in samples may interfere with measurement of reporter enzyme. Endogenous enzyme activity is reduced at the reaction pH, however, it is important to assay the level of endogenous enzyme with non-transfected cell 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 β-galactosidase activity necessitates heat inactivation, assays for luciferase and β-galactosidase should be performed individually.

Applications

Dual-Light® reporter gene assay system has been very widely used for reporter quantitation/transfection normalization from transiently transfected mammalian cell lines (3-9), and transfected primary cells (10-13). It has been used for viral studies, including gene expression regulation (14), retroviral infection (15) and viral replication (16). Dual-Light assay system has been used as a readout for both siRNA gene expression inhibition (17) and for miRNA regulation in conjunction with Applied Biosystems pMIR-REPORT™ vectors (18). Additional applications include mammalian two-hybrid analysis (19), RNA splicing assay (20), and protein-protein interaction analysis (21). Additionally, extracts from yeast cells containing a novel reporter fusion construct for measuring protein translation have been assayed (22).

II. SYSTEM COMPONENTS

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

	T1003	T1005	T1004
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
Buffer B	22 mL	3 x 22 mL	20 x 22 mL
Galacton-Plus substrate	200 µL	600 µL	4 mL
Accelerator-II	25 mL	75 mL	500 mL

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

   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 (injector not being used for Accelerator-II addition), reducing agents should be omitted. If a lysis buffer containing excess DTT has been used, the addition of hydrogen peroxide to Accelerator-II to a final concentration of 10 mM (add 1 µL of 30% H_2O_2 per 1 mL of Accelerator-II) will prevent rapid decay of signal half-life.

2. **Buffer A**: Lyophilized powder. Reconstitute in 5 mL of sterile water. Store at -20°C before reconstitution. After reconstitution, store at 4°C for 1 week or aliquot and store at -20°C.

3. **Buffer B**: Lyophilized luciferin. Reconstitute in 22 mL of sterile water. Store at -20°C before reconstitution. After reconstitution, store at 4°C for 1 week or aliquot and store at -20°C.

4. **Galacton-Plus® substrate:** 100X concentrate. Store at 4°C.

5. **Accelerator-II:** Ready-To-Use solution containing Sapphire-II™ enhancer. Store at 4˚C.

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 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 extract (supernatant) to a fresh tube. Use immediately or store at -70˚C.

B. Direct Lysis Protocol for Microplate Cultures

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

For the following hazards, see the complete safety alert descriptions in Appendix D "Safety" on page 6:

⚠ **WARNING! CHEMICAL HAZARDS. Lysis Solution.**

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 µL of Lysis Solution to each well and incubate for 10 min.
4. Continue with the Chemiluminescent Detection Protocol (Section C) omitting Step 3.

C. Chemiluminescent Detection Protocol

For the following hazards, see the complete safety alert descriptions in Appendix C "Safety" on page 6:

⚠ **WARNING! CHEMICAL HAZARDS. Buffer A, Buffer B, Galacton-Plus substrate, Accelerator-II.**

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 single day's use (100 µL/tube or well).
3. Tranfer 2 -10 µL of extracts to luminometer tubes or microplate wells (see Note 3).
4. Add 25 µL of Buffer A to each tube or well.
5. Within 10 min, inject 100 µL of Buffer B (containing Galacton-Plus substrate). 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 µL of Accelerator-II. After a 1-2 sec delay, read the β-galactosidase signal for 0.1-1 sec/well (see Note 4).

D. Protocol Notes

1. With the Dual-Light assay, the amount of cell extract assayed for each enzyme is identical, therefore the ratio of control reporter vector to experimental vector used in a transfection should be adjusted to ensure that individual enzyme signal intensities are within the detection range of the instrument used for measurement.

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 may vary depending on the level of expression and the instrumentation used. Use Lysis Solution to adjust each sample to the same volume, if necessary.

4. Signal intensities for both reporter enzyme reactions are time dependent. It is not recommended to use manual addition of Buffer B, since the luciferase reaction reaches maximum light intensity within seconds and decays rapidly. Manual addition of Accelerator-II solution is possible, as long as following recommendations are followed. Accelerator-II should be added in the same consistent time frame as the Buffer B addition, such that the incubation time with Buffer B is identical for each sample. Instruments with automatic injection will eliminate this concern. Longer or shorter measurements and delay times may be utilized but the same timing should be used when reading the β-galactosidase signal after addition of Accelerator-II.

IV. 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.
Luciferase: Prepare stock enzyme by reconstituting lyophilized luciferase (Sigma L-9506) to 1 mg/mL in 0.1 M sodium phosphate pH 7.0, 0.1% BSA. Store aliquots of stock enzyme at -80°C. Prepare serial dilutions as above. For the high end of the dilution curve, use 1-10 ng of enzyme.

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

1. 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 7.)
- 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.

2. 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.

3. 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.

4. 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

5. 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.

Specific chemical alerts

WARNING! CHEMICAL HAZARD. Accelerator II causes skin and respiratory tract irritation, and causes eye burns. Harmful if swallowed or absorbed through skin. May cause allergic reaction. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. Buffer A may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ **WARNING! CHEMICAL HAZARD. Buffer B** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ **WARNING! CHEMICAL HAZARD. Galacton-Plus substrate** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠ **WARNING! CHEMICAL HAZARD. Lysis Solution** causes eye, skin, and respiratory tract irritation. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

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For complete, updated reference list (AB #120MI08), please see http://www.appliedbiosystems.com (Product & Service Literature).