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Galacto-Light™

Galacto-Light Plus™

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

(Cat. No. BL100G, BL300G, BL100P, BL300P)
including items ABL120RG and ABL120RP

Contents **Page**

I. INTRODUCTION........ 1
II. SYSTEM COMPONENTS........ 2
III. PROTOCOL FOR β-GALACTOSIDASE DETECTION........ 2
A. Preparation of Cell Extracts........ 3
B. Detection Protocol for Tube Luminometers........ 3
C. Detection Protocol for Microplate Luminometers........ 4
Appendix A: PREPARATION OF CONTROLS........ 4
Appendix B: HEAT INACTIVATION OF ENDOGENOUS β-GALACTOSIDASE........ 5
Appendix C. USE OF SCINTILLATION COUNTERS........ 5
REFERENCES........ 6
WARRANTY........ 8

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I. INTRODUCTION

Galacto-Light™ and Galacto-Light Plus™ are chemiluminescent reporter assay systems designed for the rapid, sensitive, and non-isotopic detection of β-galactosidase in cell lysates. The Galacto-Light and Galacto-Light Plus reporter assays incorporate Galacton® and Galacton-Plus® chemiluminescent substrates for β-galactosidase with Emerald™ and Sapphire-II™ luminescence enhancers, respectively. The chemiluminescent assay has a wide dynamic range, enabling detection from 2 fg to 20 ng of β-galactosidase (1).

Galacton chemiluminescent substrate has a half-life of light emission of approximately 4.5 minutes after the addition of Light Emission Accelerator. It is suited for use with luminometers with automatic injectors and other instrumentation in which light emission measurements can be taken within a short period of time.

Galacton-Plus chemiluminescent substrate emits light which persists at a near constant level with a half-life of approximately 180 minutes after the addition of Light Emission Accelerator-II. This substrate is ideal for use with either luminometers without automatic injection capabilities or with scintillation counters. In conjunction with Light Emission Accelerator-II, Galacton-Plus shows a higher signal-to-noise ratio than Galacton.

The β-galactosidase detection assay is simple and fast. Cell lysate or purified β-galactosidase is incubated with reaction buffer for 15 minutes to 1 hour. Galacton (Galacton-Plus) substrate in the reaction buffer is cleaved by the enzyme. The sample is then placed in a luminometer and the Light Emission Accelerator is added which terminates the β-galactosidase activity and accelerates light emission.

The bacterial β-galactosidase gene is widely used as a reporter enzyme for the study of gene regulation, and more recently for identification of protein-protein interactions and in an assay for cell fusion. Chemiluminescent 1,2-dioxetane substrates for β-galactosidase, including Galacton, Galacton-Plus and Galacton-*Star*® (Galacto-*Star*™ system, Tropix BM100S and BY100S) provide highly sensitive enzyme detection (2-6) and have been utilized in reporter assays in both mammalian cell culture extracts (1,7-12) and tissue extracts (13-22), in a combined assay for luciferase and β-galactosidase activities in cell extracts (23-27; Dual-Light™ system, Tropix BD100LP) and in microinjected frog embryo extracts (28). 1,2-Dioxetane substrates have been utilized with yeast extracts (29,30), including the study of protein:protein interactions using the two-hybrid system (31-37) or DNA:protein interactions using the one-hybrid system (38), reporter gene assays in protozoan parasites (39) and in bacterial extracts (40). A novel mammalian two-hybrid system, which uses β-galactosidase peptide complementation to report protein-protein interactions, has been performed with Galacton-Plus (41,42). In addition, β-galactosidase peptide complementation has been used in a chemiluminescent assay for myoblast cell fusion in cell culture (41,43). Galacto-Light has been used in a chemiluminescent cytotoxicity assay based on release of β-galactosidase reporter enzyme from stably transfected cells (44).

When using Galacto-Light, manual injection may be performed if luminescence intensities are measured at approximately the same interval after adding the light emission accelerator to each sample. However, Galacto-Light Plus eliminates this need due to the long half-life of light emission exhibited by Galacton-Plus. The Lysis Solution included with the kit may be substituted with alternative lysis solutions and lysis procedures, if other co-transfected reporters require specific assay buffers. Alternative lysis solutions should be compared with the Galacto-Light Lysis Solution to ensure optimal assay performance.

Chemiluminescent reporter assays may be conducted in cells or tissues that have endogenous β-galactosidase activity. In this case, it is important to assay the level of endogenous enzyme with non-transfected cell extracts. Significant reductions of endogenous activity can be achieved using heat inactivation (45). Tissue extracts may also require the use of protease inhibitors (22). These procedures are described in Appendix B.

II. SYSTEM COMPONENTS

Galacto-Light™ (Cat. No. BL100G) and Galacto-Light Plus™ (Cat. No. BL100P) contain reagents sufficient for 200 single tube or 600 microplate assays. Galacto-Light™ (Cat. No. BL300G) and Galacto-Light Plus™ (Cat. No. BL300P) contains reagents sufficient for 600 single tube or 1800 microplate assays. Shelf-life for all components is one year at 4°C or as indicated on the product label.

1. **Chemiluminescent Substrate:** Galacton® or Galacton-Plus® is a 100X concentrate which is diluted in reaction buffer diluent prior to use (store at 4°C or optimally at -20°C).

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

NOTE: **Dithiothreitol** (DTT not included) **should be added fresh prior to use** to a final concentration of 0.5 mM to preserve β-galactosidase activity. However, higher concentrations of reducing agents such as β-mercaptoethanol and DTT will decrease the half-life of light emission of Galacton and Galacton-Plus. If the extended half-life of light emission from Galacton-Plus is crucial to the assay being performed, reducing agents should be omitted from the lysis solution. If lysis buffer containing excess DTT has been used, the addition of hydrogen peroxide to the Accelerator to a final concentration of 10 mM (add 1 µL of 30% H_2O_2 per 1 mL of Accelerator) will prevent rapid decay of signal half-life.

3. **Reaction Buffer Diluent**: 100 mM sodium phosphate pH 8.0, 1 mM magnesium chloride (store at 4°C).

4. **Light Emission Accelerator or Accelerator-II**: Ready-to-Use luminescence accelerator reagent (store at 4°C).

III. PROTOCOL FOR β-GALACTOSIDASE DETECTION

A. Preparation of Cell Extracts From Tissue Culture Cells

1. Aliquot the required amount of **Lysis Solution. Add fresh DTT to 0.5 mM.**

2. Rinse cells 2 times with 1X Phosphate Buffered Saline (PBS).

3. Add **Lysis Solution** to cover the cells (250 µl of Lysis Buffer for a 60 mm culture plate should be adequate).

4. Detach cells from culture plate using a rubber policeman or equivalent.

   Non-adherent cells should be pelleted and lysis buffer should be added sufficient to cover the cells. The cells should then be resuspended in the lysis buffer by pipetting.

5. Transfer cells to a microfuge tube and centrifuge for 2 minutes to pellet any debris.

6. Transfer supernatant to a fresh microfuge tube. Cell extracts may be used immediately or frozen at -70°C for future use.

III. PROTOCOL FOR β-GALACTOSIDASE DETECTION (cont.)

B. Detection Protocol for Tube Luminometers

All assays should be performed in triplicate.

1. Dilute **Galacton (Galacton-Plus) substrate** 1:100 with **Reaction Buffer Diluent** to make **Reaction Buffer.** This mixture will remain stable for several months if stored uncontaminated at 4°C. It is recommended to only dilute the amount of substrate that will be used within a two month period.

2. Equilibrate the required volume of **Reaction Buffer** to room temperature.

3. Aliquot 2 to 20 µl of individual cell extracts into luminometer sample tubes. If using less than 20 µl of extract, lysis solution should be added to bring the total volume up to 20 µl.

NOTE: **The amount of cell extract required may vary depending on the amount of expression and the instrumentation used. Use 5 µl of extract for positive controls and 10 to 20 µl of extract for experiments with potentially low levels of enzyme.**

4. Add 200 µl of **Reaction Buffer** to each luminometer tube and gently mix. Incubate at room temperature for 60 minutes. Incubations can be as short as 15 minutes, but the linear range of the assay may decrease.

NOTE: **Measurements are time dependent. Reaction Buffer should be added to sample extracts in the same time frame as they are counted on the luminometer. For example, if it takes 10 seconds to completely count a sample, then Reaction Buffer should be added to tubes every 10 seconds.**

5. Place tube in a luminometer. Inject 300 µl of **Light Emission Accelerator**. After a 2 to 5 second delay following injection, count the sample for 5 seconds. If manual injection is used, the Accelerator should be added in the same consistent time frame as the Reaction Buffer is added. This is critical when using Galacton.

NOTE: **Reaction components are scaled down if a single tube luminometer with a smaller volume injector is used, however, sensitivity may be affected slightly. For example, if a 100 µl injector is used, the reaction can be scaled back using 2-10 µl of lysate, 70-100 µl of Reaction Buffer, and 100 µl of Light Emission Accelerator.**

III. PROTOCOL FOR β-GALACTOSIDASE DETECTION (cont.)

C. Detection Protocol for Microplate Luminometers

All assays should be performed in triplicate.

1. Dilute **Galacton (Galacton-Plus) substrate** 1:100 with **Galacto-Light Reaction Buffer Diluent** to make **Reaction Buffer.** This mixture will remain stable for several months if stored uncontaminated at 4°C. It is recommended to only dilute the amount of substrate that will be used within two months.

2. Equilibrate the required amount of **Reaction Buffer** to room temperature.

3. Aliquot 2 to 20 µl of individual cell extracts into microplate wells. If using less than 20 µl of extract, lysis solution should be added to bring the total volume up to 20 µl.

NOTE: **The amount of cell extract required may vary depending on the amount of expression and the instrumentation used. Use 5 µl of extract for positive controls and 10 to 20 µl of extract for experiments with potentially low levels of enzyme.**

4. Add 70 µl of **Reaction Buffer** to each microplate well and gently mix. Incubate at room temperature for 60 minutes. Incubations can be as short as 15 minutes, but the linear range of the assay may decrease.

NOTE: **Measurements are time dependent. Reaction Buffer should be added to sample extracts in the same time frame as they are counted on the luminometer. For example, if it takes 10 seconds to completely count a sample, then Reaction Buffer should be added to tubes every 10 seconds.**

5. Place microplate in a luminometer. Inject 100 µl of **Light Emission Accelerator**. After a 1 to 2 second delay following injection, count each sample for 1-5 seconds. If manual injection is used, the Accelerator should be added in the same consistent time frame as the Reaction Buffer is added. If manual injection is used with Galacton-Plus®, the entire plate can be read with a reading time of 0.1-1 second per sample.

APPENDIX 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, 1.0% BSA. Store at 4°C. A standard curve can be generated by serially diluting the stock enzyme in Galacto-Light Lysis Buffer containing 0.1% BSA. 2-20 ng of enzyme should be used for the high end detection limit. Proceed with Detection Protocol (Section III.B or III.C).

Negative Control

Assay a volume of mock transfected cell extract equivalent to the volume of experimental cell extract used. Proceed with Detection Protocol (Section III.B or III.C).

APPENDIX B: HEAT INACTIVATION OF ENDOGENOUS β-GALACTOSIDASE

Some cell lines may exhibit relatively high levels of endogenous β-galactosidase activity. This may lead to background which will decrease the overall sensitivity of the assay by lowering the signal to noise ratio. A procedure for heat inactivation of endogenous β-galactosidase activity has been described (45). A modified version of this protocol has also been described (22) in which a cocktail of protease inhibitors is used in conjunction with the heat inactivation procedure for reducing β-galactosidase in tissue extracts. Both are summarized below.

The following procedures should be performed immediately prior to the Detection Protocol (Section III.B or III.C).

Inactivation of β-Galactosidase Activity in Cell Extracts

1. Following cell extract preparation, heat the extract to 48°C for 50 minutes.

2. Proceed with Detection Protocol (Section III.B or III.C).

NOTE: Although Young *et al.* (45) suggest 50°C for 60 minutes, we suggest heat inactivation at 48°C for 50 minutes.

Inactivation of Endogenous β-Galactosidase Activity in Tissue Extracts

1. To the Galacto-Light lysis buffer, add PMSF to a final concentration of 0.2 mM and leupeptin to a final concentration of 5 μg/ml just before use.

2. Heat inactivate extracts by heating at 48°C for 60 minutes.

3. Proceed with Detection Protocol (Section III.B or III.C).

NOTE: AEBSF (Sigma A-5938) may be used in place of PMSF (Sigma P-7626). AEBSF is a water soluble serine protease inhibitor. Leupeptin (Sigma L-2884) is recommended.

APPENDIX C: USE OF SCINTILLATION COUNTERS

A liquid scintillation counter may be used as a substitute for a luminometer, however, sensitivity may be lower (46,47). When using a scintillation counter, it is necessary to turn off the coincident curcuit to measure chemiluminescence directly (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 curcuit, a linear relationship can be established by taking the square root of the counts per minute measured and subtracting the instrument background.

$$\text{Actual} = (\text{measured-instrument background})^{1/2}$$

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