User Manual

Luminescent β-gal User Manual

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Cat. Nos. 631712 & 631713
PT2106-1 (032712)

Table of Contents

List of Figures

Contact Us For Assistance	
Customer Service/Ordering:	**Technical Support:**
Telephone: 800.662.2566 (toll-free)	Telephone: 800.662.2566 (toll-free)
Fax: 800.424.1350 (toll-free)	Fax: 650.424.1064
Web: www.clontech.com	Web: www.clontech.com
E-mail: orders@clontech.com	E-mail: tech@clontech.com

I. Introduction

The *lacZ* gene is commonly used as a reporter gene for the analysis of heterologous promoters and enhancers in both stably and transiently transfected eukaryotic cells (Alam *et al.*, 1990). In addition, expression of β-galactosidase, under the control of a constitutive promoter (such as SV40), is frequently used as an internal control when transfected with other vectors to normalize variability, which may arise due to variation in transfection efficiency or cell extract preparation. These systems utilize Galacton-Star as the chemiluminescent substrate for β-galactosidase (Bronstein *et al.*, 1989). Galacton-Star greatly improves the utility of β-galactosidase as a transcriptional reporter by increasing the sensitivity of β-galactosidase assays, extending the linear range of detection, and simplifying the assay procedure. Whereas conventional colorimetric and fluorometric assays have detection limits of 1 x 10^9 and 1 x 10^6 molecules, respectively, the Luminescent β-galactosidase assay can detect as few as 1 x 10^4 molecules (equivalent to 10^{-13} g).

Figure 1 outlines the chemiluminescent β-galactosidase assay. First, cell lysates are prepared from cells transfected with the appropriate β-galactosidase reporter construct. Second, the Reaction Substrate (which contains Galacton-Star) is added to the Reaction Buffer. Then each lysate is incubated with buffer for 60 minutes. Cleavage of a galactoside moiety, from Galacton-Star by β-galactosidase, yields a dioxetane anion that further degrades with the concurrent production of light (Bronstein *et al.*, 1989). The light emission can be used as a quantitative measure of β-galactosidase activity. The Reaction Buffer, which is specially formulated to suppress the activity of eukaryotic β-galactosidase, has minimal effect on the activity of the bacterial enzyme expressed from reporter plasmids. Thus, vector-encoded bacterial β-galactosidase may be specifically assayed in cells expressing endogenous β-galactosidase. The Reaction Buffer contains an enhancer that amplifies the chemiluminescent light signal. Because the Reaction Substrate produces light signals that are stable over several hours, detection can be performed in scintillation counters or in luminometers not equipped with automatic injectors.

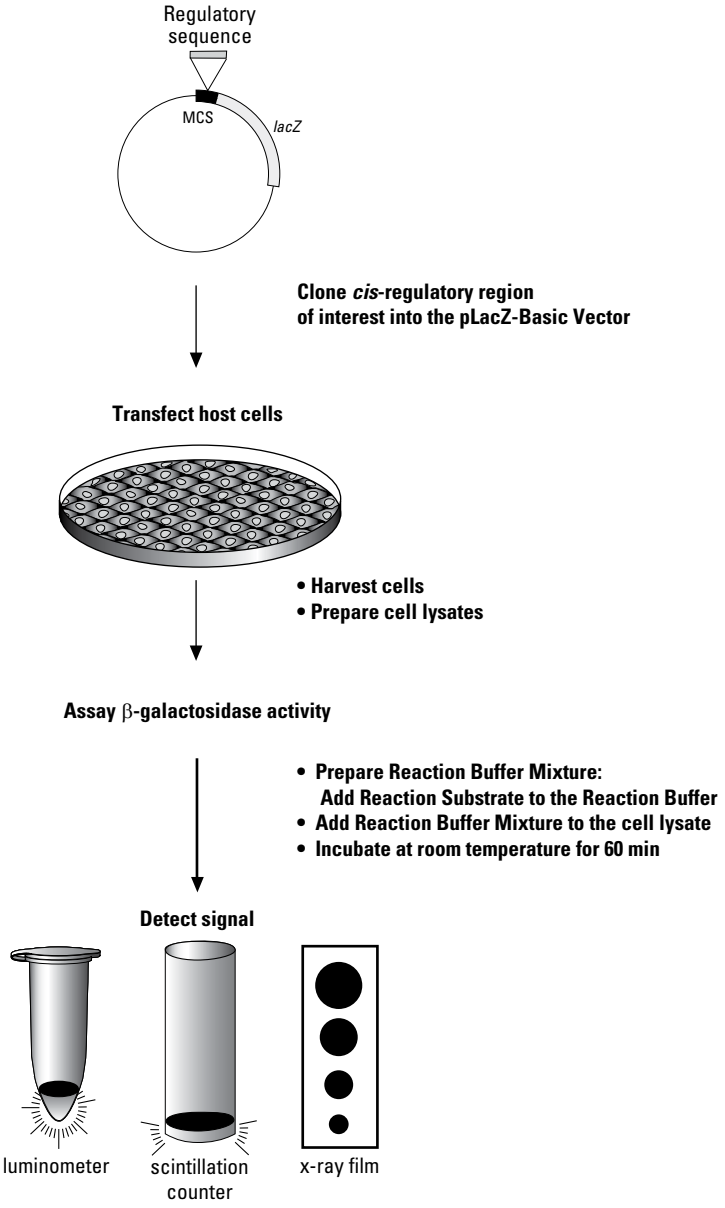


Figure 1. Flow chart of a chemiluminescent β-galactosidase assay.

I. Introduction continued

Clontech's pLacZ Reporter Vectors

The Luminescent β-galactosidase Reporter System 3 includes two pLacZ vectors, which have been designed to provide maximal flexibility in studying regulatory sequences of interest. For vector maps and multiple cloning site (MCS) sequences, see Appendix B.

- **pLacZ-Basic** lacks eukaryotic promoter and enhancer sequences, and it has an MCS that allows promoter DNA fragments to be inserted upstream of the *lacZ* gene. Enhancers can be cloned into either the MCS or unique downstream sites. The lacZ coding sequences are followed by an intron and polyadenylation signal from SV40 to ensure proper and efficient processing of the *lacZ* transcript in eukaryotic cells. A second polyadenylation signal upstream of the MCS reduces background transcription (Araki *et al*., 1988).
- **pLacZ-Control** is pLacZ-Basic with the SV40 early promoter inserted upstream of the *lacZ* gene and the SV40 enhancer (derived from the SV40 early promoter) inserted downstream. pLacZ-Control should express *lacZ* in most cell types and provides an important positive control in most experiments.

Although the pLacZ vectors were designed for use with Clontech's chemiluminescent β-galactosidase system, these vectors are compatible with any β-galactosidase assay.

II. List of Components

Store the pLacZ vectors and positive control β-galactosidase at –20°C. Store Reaction Buffer and Substrate at 4°C. The Reaction Buffer and Substrate are light sensitive; store them in the dark or in an amber bottle.

The **Luminescent β-galactosidase Reporter System 3** (Cat. No. 631713) contains sufficient reagents for 100 chemiluminescent β-galactosidase assays, and the **Luminescent β-galactosidase Detection Kit II** (Cat. No. 631712) contains enough reagents for 300 chemiluminescent β-galactosidase assays.

Reporter System 3	Detection Kit II	
Cat. No. 631713	Cat. No. 631312	
10 µg	–	pLacZ-Basic Vector
10 µg	–	pLacZ-Control Vector
25 ml	70 ml	Reaction Buffer
500 µl	1.4 ml	Reaction Substrate
30 µl	50 µl	Positive Control β-galactosidase (1,000 units/ml in 100 mM Na_2HPO_4 (pH 7.4) and 50% (v/v) glycerol.)

III. Additional Materials Required

The following reagents are required but not supplied.

- **1.0 M Dithiothreitol** (DTT; 1,000X stock)
  Add 1.54 g of DTT to 8 ml of ddH_2O.
  Mix gently until dissolved. Adjust final volume to 10 ml with ddH_2O.
  Aliquot into microcentrifuge tubes and freeze at –20°C.
  Stable for 1 month.

- **100 mM Na_2HPO_4**
  1.43 g to a final volume of 100 ml with ddH_2O. Store at room temperature.

- **100 mM NaH_2PO_4**
  1.2 g to a final volume of 100 ml with ddH_2O. Store at room temperature.

- **100 mM K_2HPO_4**
  1.74 g to a final volume of 100 ml with ddH_2O. Store at room temperature.

- **100 mM KH_2PO_4**
  1.36 g to a final volume of 100 ml with ddH_2O. Store at room temperature.

- **Phosphate-buffered saline** (PBS; pH 7.4)
  Add each of the following to 1.8 L of ddH_2O:

		Final Concentration
16.5 g	Na_2HPO_4	58 mM
4.1 g	NaH_2PO4	17 mM
8.0 g	NaCl	68 mM

  Adjust to pH 7.4 with 0.1 N NaOH. Add ddH_2O to a final volume of 2 L.
  Store at room temperature. Chill to 0–4°C prior to preparation of cell lysates.

- **Lysate buffer** (100 mM potassium phosphate, pH 7.8)
  9.15 ml 100 mM K_2HPO_4
  0.85 ml 100 mM KH_2PO_4
  10.00 µl 1.0 M stock DTT (1 mM final)
  Add DTT just prior to use. Keep on ice until ready for use.

- **Enzyme dilution buffer** (100 mM sodium phosphate, pH 7.4)
  8.1 ml 100 mM Na_2HPO_4
  1.9 ml 100 mM NaH_2PO_4
  0.1 g BSA (1% final)
  Store at 4°C.

- **Luminometer**

  Assays are best monitored with a tube or plate luminometer. If using a plate luminometer, perform the assays in an opaque 96-well microtiter plate and have a multichannel pipette available.

  Assays can also be monitored via exposure of the reactions (in an opaque white microtiter plate) to x-ray film; however, this method is several orders of magnitude less sensitive than using a luminometer or scintillation counter. Therefore, the x-ray film method is well-suited for qualitative, rather than quantitative, results.

The following items are necessary if monitoring chemiluminescence with either a plate luminometer or x-ray film.

- **White, opaque 96-well microtiter plates**
- **Multichannel pipette**

IV. Chemiluminescent β-Galactosidase Protocols

PLEASE READ ENTIRE PROTOCOL BEFORE BEGINNING

A. Proper Use of Controls

1. Negative controls

   A negative control is necessary to determine the level of background signals associated with the cell lysates. The best negative control is 10–30 µl of a cell lysate from cells transfected with the pLacZ-Basic Vector, which lacks a eukaryotic promoter and enhancer. Alternatively, use a cell lysate from untransfected cells. The averaged value obtained for three negative controls should be subtracted from each experimental result.

2. Positive controls

   a. Positive control for transfection and expression of exogenous DNA

      A positive control is necessary to confirm transfection and expression of exogenous DNA and to verify the presence of active bacterial β-galactosidase in the cell lysates. Expression of functional β-galactosidase in transfected cells can be confirmed by assaying 10–30 µl of cell lysates obtained from cells transfected with the pLacZ-Control Vector, which contains the *lacZ* structural gene under transcriptional control of the SV40 promoter and enhancer. Cells transfected with this plasmid should yield high activity (100–500 RLU using a Turner Designs Model 20e luminometer) within 48–72 hr after transfection. We recommend transfecting with pLacZ-Control in triplicate.

   b. Positive control for method of detection

      The positive control bacterial β-galactosidase can be used to confirm that the detection method is working. To do this, simply add 1–2 µl of the positive control bacterial β-galactosidase to cell lysates obtained from untransfected cells. This should give a strong positive signal.

      A dilution series of the positive control enzyme can also be used to determine the linear range of the assay.

3. Normalizing transfection efficiencies

   Observed values of β-galactosidase activity should be normalized with a second reporter to eliminate variability resulting from differences in transfection efficiency or preparation of cell extracts (Alam *et al.*, 1990; Sambrook *et al.*, 1989). See also Section IV.B.2.b.

B. Transfection of Mammalian Cells with β-galactosidase Expression Vectors

1. Transfection techniques

   The β-galactosidase expression vectors may be transfected into eukaryotic cells by a variety of techniques. Clontech recommends using the CalPhos™ Mammalian Transfection Kit (Cat. No. 631312) or our high-efficiency transfection reagent, Xfect™ (Cat. Nos. 631317 & 631318). The transfection method of choice depends primarily on the type of cell being transfected. Different cell lines may vary by several orders of magnitude in their ability to take up and express exogenously added DNA. Moreover, a method that works well for one type of cultured cell may be inferior for another. When working with a cell line for the first time, compare the efficiencies of several transfection protocols using pLacZ-Basic and pLacZ-Control Vectors as described in Section IV.A.

2. Transfection considerations

   a. Perform transfections in triplicate

      Each different vector construct should be transfected (and subsequently assayed) in triplicate to minimize variability among treatment groups. The primary sources of such variability are differences in transfection efficiencies (see below) and differences in recovery of β-galactosidase in cell lysates.

IV. Chemiluminescent β-Galactosidase Protocols continued

b. Normalize for transfection efficiency

When using a reporter to monitor the effects of promoter and enhancer sequences on gene expression, the level of reporter gene expression should be normalized to eliminate variability that may arise from differences in transfection efficiency or preparation of cell extracts (Alam *et al.*, 1990; Sambrook *et al.*, 1989). This is easily done by cotransfecting a control plasmid that constitutively expresses an enzymatic activity, which can be readily distinguished from β-galactosidase (e.g., luciferase). The level of the second enzymatic activity can then be used to normalize the levels of β-galactosidase among different treatment groups:

$$\text{Normalized β-gal activity} = \frac{\text{Observed β-gal activity}}{\text{Control reporter activity}}$$

NOTE ON EFFECTS OF SV40 LARGE T-ANTIGEN (COS CELLS): The specific level of expression for the pLacZ vectors is likely to vary for different cell types. This may be particularly true for COS cells and other cell lines that express the SV40 large T-antigen. The large T-antigen promotes replication of the SV40 origin, sequences that are found in the promoter region of the pLacZ-Control Vector. The combination of the large T-antigen and SV40 origin leads to a higher copy number of these vectors in COS cells, which in turn may result in increased expression of the *lacZ* reporter gene relative to vectors lacking the SV40 origin.

C. Preparation of Cell Lysates for β-galactosidase Assay

For transient transfection assays, maximal levels of β-galactosidase are generally detected 48–72 hr after cell transfection. This range is suggested only as a starting point, as optimal times for collecting samples will vary with different cell types, cell densities, and other experimental conditions. An alternate procedure using detergent lysis is described in Appendix A. This procedure may be more suitable for certain cell types.

The volumes listed below are for cells cultured in 60-mm tissue culture plates. If a larger or smaller surface is used for transfecting adherent cells, the volumes should be adjusted accordingly.

The procedure below describes harvesting adherent cells. If working with suspended cell cultures, simply collect an equivalent cell mass by centrifugation, wash three times by resuspending in 500 µl of ice-cold PBS followed by centrifugation, and proceed from Step IV.C.6.

1. Wash cells on the plate twice with 4.0 ml of ice-cold PBS.
2. Add 1.0 ml of ice-cold PBS to each plate.
3. Scrape cells off the plate using a rubber policeman (or equivalent), and transfer cell suspension to a microcentrifuge tube on ice.
4. If residual cells are still on the plate, use another 500 µl of PBS to collect the remaining cells, and transfer them to the same microcentrifuge tube.
5. Centrifuge the cells at maximum speed for 15 sec.
6. Aspirate the supernatant, being careful not to disturb the cell pellet.
7. Gently resuspend cells in 1.0 ml of ice-cold PBS.
8. Repeat Steps 5 and 6, and carefully aspirate the last traces of PBS.

   NOTE: The cell pellet can be stored at –20°C for 1–3 days with minimal loss of β-galactosidase activity.
9. Gently resuspend the cell pellet in 75 µl of ice-cold lysate buffer.
10. Place the tube with cell suspension in a dry-ice/ethanol bath for 1 min (or until completely frozen).
11. Thaw the cell suspension at 37°C for 1–2 min.
12. Repeat the freeze/thaw cycle (Steps 10–11) twice.
13. Centrifuge at maximum speed for 5 min at 4°C.
14. Transfer the supernatant to a fresh tube, and keep it on ice. This is the "cell lysate" that is assayed in Section D, below.

    NOTE: The cell lysates may be assayed immediately for β-galactosidase or stored at –70°C until use.

IV. Chemiluminescent β-Galactosidase Protocols continued

D. Chemiluminescent Detection of β-galactosidase

It is important to stay within the linear range of the assay. High intensity light signals can saturate the photomultiplier tube in luminometers, resulting in false low readings. In addition, low intensity signals that are near background levels may be outside the linear range of the assay. See the Troubleshooting Guide (Section VI) for more information on determining the linear range and adjusting the amount of lysate used to bring the signal within this range. Figure 2 in the Troubleshooting Guide gives an example of linear range determination.

IMPORTANT: Before you start, prepare the Reaction Buffer Mixture.

- Warm enough Reaction Buffer and Reaction Substrate for the entire experiment to room temperature.
- Add 4 µl of Reaction Substrate to 196 µl of Reaction Buffer. Note, you will need 200 µl for each reaction; therefore, prepare a master Reaction Buffer Mixture for the appropriate number of assays you wish to perform. For example, to prepare enough buffer for two reactions, add 8 µl of Reaction Substrate to 392 µl of Reaction Buffer.

1. Chemiluminescent β-galactosidase assay
   We recommend transfecting and subsequently assaying each construct in triplicate. Average the results; if the readings vary dramatically, repeat the assay.

   a. Aliquot 20–30 µl of individual cell lysates into sample tubes (or into wells of a white, opaque 96-well, flat-bottom microtiter plate).

      NOTE: The amount of cell extract required may vary depending upon the amount of expression and the detection device used. Use 10–30 µl of extract for positive controls and 20–30 µl of extract for experimental conditions with potentially low levels of β-galactosidase. It is important to vary the amount of extract to keep the signal within the linear range of the assay. **Remember to correct for individual sample volumes when tabulating final results.**

   b. Add 200 µl of the Reaction Buffer Mixture to each cell lysate and mix gently.

   c. Incubate at room temperature (20–25°C) for 60 min.

      NOTE: Light signals produced during the 60-min incubation are stable for >1 hr; therefore, detection can be performed 0–60 min after the incubation.

2. Proceed with one of the following detection methods:

   - **Tube luminometer**
     If the assay is performed in a tube suitable for a luminometer, place the sample directly in the instrument; otherwise, transfer the sample to a luminometer tube and place it in the instrument. Record light emission as 5-sec integrals.
   - **Plate luminometer**
     The entire assay may be performed in white, 96-well flat-bottom microtiter plates suitable for plate luminometers. Record light signals as 5-sec integrals.
   - **Scintillation counter**

     a. Transfer the entire solution to a 0.5-ml Eppendorf tube.

        NOTE: We highly recommend using scintillation counter adapters, which are designed to keep samples upright.

     b. Place the tube into the washer of the scintillation counter adapter, and place the adapter in the scintillation rack. Set the integration time for at least 15 sec.

        NOTE: Integration times less than 15 sec may not produce accurate results.

     c. To detect chemiluminescent signals, use a single photon count program on the scintillation counter. Consult your scintillation counter manufacturer for further information about photon single count software.

IV. Chemiluminescent β-Galactosidase Protocols continued

- **X-ray film**

  Light emission can also be recorded by exposure of x-ray film to white, opaque 96-well, flat-bottom microtiter plates. The resulting spots on the film can be quantitated by comparison to positive and negative control incubations. Note that x-ray film is several orders of magnitude less sensitive than a luminometer or scintillation counter.

  Overlay the microtiter plate with x-ray film, cover the film with plastic wrap, and place a heavy object such as a book on top to hold the film in place. Expose the film at room temperature for 5–30 min.

NOTE: To compare samples accurately, you must remain within the linear response capability of the x-ray film. We therefore recommend that you obtain several different exposures.

V. Normalizing Transfection Efficiencies

When using a reporter to monitor the effects of promoter and enhancer sequences on gene expression, the observed values of reporter gene expression must be normalized to eliminate variability that may arise from differences in transfection efficiency or cell extract preparation (Alam *et al.*, 1990; Sambrook *et al.*, 1989). This is typically done by cotransfecting a second plasmid that constitutively expresses an enzyme activity, which can be readily distinguished from the primary reporter molecule. For example, in an experiment using chloramphenicol acetyl transferase (CAT) as the reporter molecule, standard deviations of CAT activity were reduced nearly three-fold when β-galactosidase was used to normalize for transfection efficiency (Alam *et al.*, 1990).

The Luminescent β-galactosidase Detection Kit II and the plasmid pLacZ-Control, which constitutively expresses β-galactosidase in most eukaryotic cells, can be used to normalize transfection efficiencies in experiments that use other reporters.

To normalize for transfection efficiency, cotransfect a constant amount of pLacZ-Control with each experimental construct, and then assay β-galactosidase each time the primary reporter enzyme is assayed. Use the following equation to normalize the data from each sample:

$$\text{Normalized β-gal activity} = \frac{\text{Observed β-gal activity}}{\text{Control reporter activity}}$$

VI. Troubleshooting Guide

A. Determining Linear Range of the Assay

Prepare and assay a dilution series using the Positive Control β-galactosidase provided with the kit. Figure 2 shows light readings from approximately 2–6,000 RLU are within the linear range of the assay. In this example, the linear range corresponds to approximately 10^{-12} to 10^{-7} g of the Positive Control β-galactosidase or approximately 10^{-6} to 10^{-1} relative units of β-galactosidase activity.

NOTE: Figure 2 is only an example; therefore, the linear range should be independently determined for each luminometer and each set of experimental conditions.

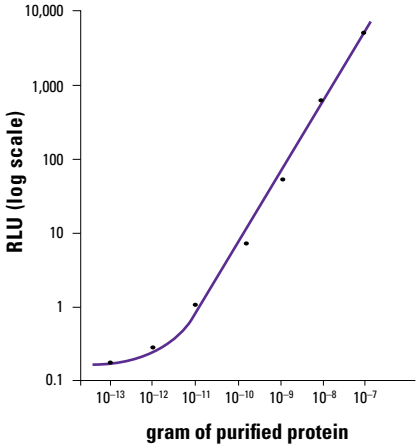


Figure 2. Determining the linear range of the assay. Serial dilutions of the positive control β-galactosidase were prepared and assayed as described in the protocol using a Turner Model TD-20e luminometer with a 5-sec integration time. Background signals from samples containing no β-galactosidase have been subtracted from all values. RLU = Relative Light Units.

B. Little or no Signal from Transfected Cells

There are several possible explanations for a weak or nondetectable signal in the chemiluminescence assay. These include the following:

- The detection method is not working.
- Cells are not being fully lysed during preparation of cell lysates.
- Cells are not taking up and/or expressing the DNA.

The following procedures should help determine which of the explanations is causing a low signal:

1. To ensure that your detection method is working, use the bacterial β-galactosidase provided with the kit as a positive control (as described in Section IV.A.2).
2. To determine whether the cell lysis procedure has been successful, examine a small amount of the cell suspension from Step IV.C.11 under a microscope. Staining the cells with trypan blue may be necessary. The presence of a substantial number of intact cells (>20%) indicates incomplete lysis. In this case, repeat the freeze/thaw cycle (Sections IV.C.10–11) twice. Alternatively, use the detergent lysis procedure described in Appendix A. The alternative protocol may result in better recovery of β-galactosidase from some cell types.
3. To ensure that the transfection is working and that the cells are expressing the transfected DNA, include a positive control for transfection using the pLacZ-Control vector. If the positive control transfection does not produce a strong signal, use the pLacZ-Control vector to optimize transfection and expression of β-galactosidase in your cell system.

VI. Troubleshooting Guide continued

4. If the signal is still weak, try the following modifications:
   a. Increase the number of cells used in transfections by using a larger diameter plate for adherent cells or by increasing the volume of media for suspension cultures.
   b. If background signals obtained from negative controls (i.e., untransfected cells or cells transfected with pLacZ-Basic) are low, increase the volume of cell lysate assayed from experimental cultures in Step IV.D.1.b.
   c. Increase the post-transfection interval prior to the preparation of cell lysates.
   d. If using a tube or plate luminometer to monitor chemiluminescence, refer to the instrument instructions for means of increasing the sensitivity of light detection.
   e. If using exposure of x-ray film to monitor chemiluminescence, increase the exposure time.
   f. Try an alternative method of transfection to increase the number of transfected cells.

C. Excessive Background Signal

1. If the signal from experimental samples is strong, decrease the amount of cell lysate assayed in Step IV.D.1 or dilute the cell lysate using 1X lysate buffer.
2. If negative controls give a positive signal, then excessive background is probably due to endogenous β-galactosidase activity. To reduce endogenous β-galactosidase activity, pretreat the cell lysates at 50°C (Young *et al.*, 1993). This will further suppress any endogenous β-galactosidase activity, while having little or no effect on the bacterial enzyme expressed by the pLacZ vectors (or on the Positive Control β-galactosidase provided in the kit). A modified protocol for the chemiluminescent detection of β-galactosidase beginning at Section IV.D.1.a is as follows:
   a. Warm enough Reaction Buffer and Substrate to room temperature for the entire experiment, and then prepare a master Reaction Buffer Mixture.
   b. Aliquot 10–30 µl of individual cell lysates into sample tubes.
   c. Incubate the cell lysates at 50°C for 1 hr using a heat block or water bath.
   d. Cool the cell lysates to room temperature by placing briefly on ice, and then placing at room temperature.
   e. Continue with the detection protocol beginning at Step IV.D.1.b.

D. Signal Exceeds Linear Range of the Assay

This problem is easily corrected either by assaying a lower volume of cell lysate at Section IV.D.1.a or by diluting the samples.

E. Excessive Variation Among Triplicate Assays

Different values for triplicate samples of the same experimental condition may be due to variations in the preparation of triplicate samples. Possible sources of such variation could include differences in the quantity of DNA or number of cells used for transfection, differences in transfection efficiency, and varying recovery of β-galactosidase from cell lysates. Most of this variability can be eliminated by normalizing with a second reporter (see Section IV.B.2.b).

VII. References

Alam, J. & Cook, J. L. (1990) Reporter genes: Application to the study of mammalian gene transcription. *Anal. Biochem.* **188**:245–254.

Araki, E., Shimada, F., Shichiri, M. & Ebina, Y. (1988) pSV00CAT: low-background CAT plasmid. *Nucleic Acids Res.* **16**:1627.

Bronstein, I., Edwards, B. & Voyta, J. C. (1989) 1,2-dioxetanes: Novel chemiluminescent enzyme substrates: Applications to immunoassays. *J. Chemilumi. Biolum.* **4**:99–111.

Chen, C. & Okayama, H. (1988) Calcium phosphate mediated gene transfer: A highly efficient transfection system for stably transforming cells with plasmid DNA. *BioTechniques* **6**:632–638.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Sinai, P., Kondepudi, A., Yang, T., Adams, M., Kitts, P. & Kain, S. (October 1994) A chemiluminescent assay for β-galactosidase: Application to the analysis of *cis*-regulatory DNA elements. *Clontechniques* **IX**(4):1–4.

Young, D. C., Kingsley, S. D., Ryan, K. A. & Dutko, F. J. (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.

Appendix A: Alternate Cell Lysis Protocol

The following cell lysis procedure (Young *et al.*, 1993) may result in more complete lysis of some cell types than the freeze/thaw method described in Section IV.C and may thus yield higher levels of recovered β-galactosidase activity.

Additional materials required

- Detergent lysis solution
  (100 mM potassium phosphate [pH 7.8] + 0.2% Triton X-100)

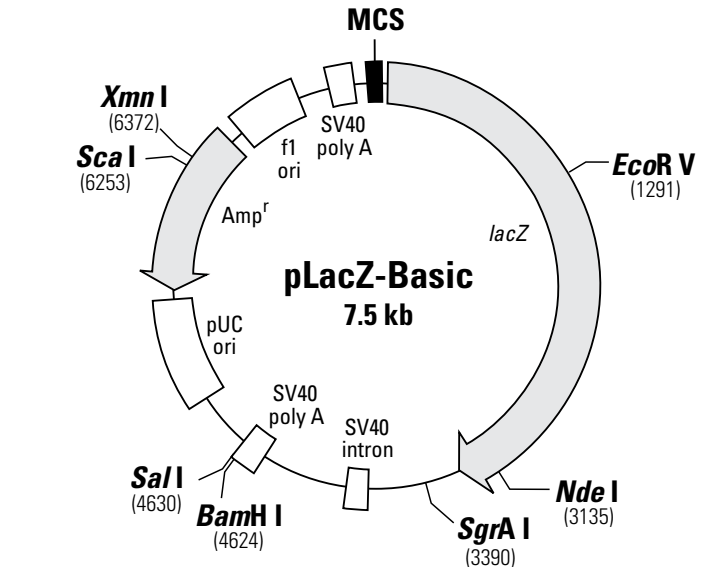
9.15	ml	100 mM K_2HPO_4
0.85	ml	100 mM KH_2PO_4l
20.0	µl	Triton X-100 (0.2% final)
*10.0	µl	1,000X stock DTT (1 mM final)

*Add DTT just prior to use. Keep on ice.

Procedure

1. Warm enough detergent lysis solution for all your samples to room temperature (22–25°C).
2. Rinse cells twice with room-temperature PBS.
3. Add sufficient detergent lysis solution to cover the cells (250 µl should be adequate for a 60-mm tissue culture plate).
4. Detach cells from the tissue culture plate using a rubber policeman or equivalent, and transfer them to a microcentrifuge tube.
5. Mix gently for 1 min, and incubate at room temperature for 5 min.
6. Centrifuge at maximum speed for 2 min to pellet any debris.
7. Transfer supernatant to a fresh microcentrifuge tube.
8. Cell lysates may be assayed immediately for β-galactosidase activity (Section IV.D) or stored at –70°C until use.

Appendix B: Plasmid Maps & Cloning Sites



1 10 20 30 40 50

CCCGGGAGGTACCGAGCTCTTACGCGTGCTAGCTCGAGATCTAAGTAAGCTT

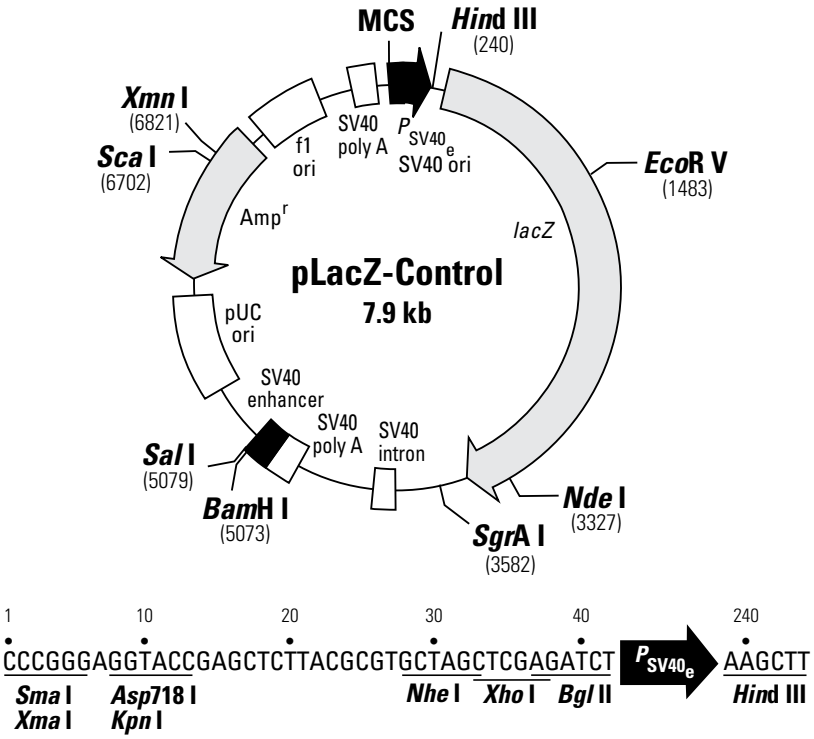
Sma I / *Xma* I — *Asp*718 I / *Kpn* I — *Nhe* I — *Xho* I — *Bgl* II — *Hin*d III

pLacZ-Basic lacks eukaryotic promoter and enhancer sequences. The MCS allows promoter DNA fragments to be inserted upstream of the *lacZ* gene. Enhancers can be cloned into either the MCS or unique downstream sites. Restriction sites shown on the map are unique.

pLacZ-Basic and pLacZ-Control Vectors contain an SV40 intron and the SV40 polyadenylation signal inserted downstream of the β-galactosidase coding sequences to ensure proper and efficient processing of the transcript in eukaryotic cells. A second polyadenylation signal upstream of the MCS reduces background transcription (Araki *et al.*, 1988). The vector backbone contains an f1 origin for single-stranded DNA production and a pUC19 origin of replication and an ampicillin resistance gene for propagation in *E. coli*. The multiple cloning site region is identical in both vectors except for a 202-bp promoter fragment that has been inserted between the Bgl II and Hind III sites in pLacZ-Control.

The sequence of pLacZ-Basic has been deposited in GenBank (Accession No. U13184). Complete sequence information can be downloaded from our web site at **www.clontech.com/manuals**.

Appendix B. Plasmid Maps & Cloning Sites continued



pLacZ-Control is pLacZ-Basic with the SV40 early promoter inserted upstream of the *lacZ* gene and the SV40 enhancer (derived from the SV40 early promoter) inserted downstream. pLacZ-Control should express β-galactosidase in most cell types and provides an important positive control in most experiments. Restriction sites shown on the map are unique.

See the description of pLacZ-Basic (previous page) for information about elements common to both vectors.

The sequence of pLacZ-Control has been deposited in GenBank (Accession No. U13186). Complete sequence information can be downloaded from our web site at **www.clontech.com/manuals**.