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A new lysis buffer for luciferase, CAT and beta-galactosidase reporter gene co-transfections

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A new lysis buffer formulation allows assays of luciferase, CAT and beta-galactosidase reporter activity to be conveniently performed with the same cell extract. The new Reporter Lysis Buffer gives reporter enzyme activities comparable to or better than those obtained with the standard lysis methods for these reporter genes.

Introduction

Transcriptional regulation of defined genomic sequences within cells can be studied following *in vitro* transfection of cultured cells with reporter genes. This powerful and dynamic approach often involves transfecting cells with DNA engineered to include regulatory sequences of interest, such as promoter or enhancer sequences, fused to plasmid DNA encoding a reporter gene. Transfected cells transcribe the reporter gene into RNA, which is subsequently translated into the reporter enzyme protein. Cell lysates are prepared and assayed for the appropriate reporter enzyme activity. Three reporter genes, coding for luciferase, beta-galactosidase and CAT (chloramphenicol acetyltransferase), are commonly used because their corresponding enzymes are easy to assay and are either absent from nontransfected cells or present only at low levels.

Many customers have requested a cell lysis reagent which would allow convenient assays of multiple reporter enzyme activities. This would be useful for assaying cells co-transfected, for example, with any combination of the luciferase, beta-galactosidase or CAT reporter genes. Unfortunately, no one lysis procedure allowed high activity assays of all three enzymes. Promega's new Reporter Lysis Buffer was specifically developed to lyse cultured mammalian cells and to be compatible with the luciferase, CAT and beta-galactosidase enzyme assays.

Disadvantages of standard freeze/thaw lysis

In the standard freeze/thaw method used to prepare cell lysates for CAT and beta-galactosidase activity assays, cells are washed in standard PBS (phosphate buffered saline) and then incubated for 5 minutes in a Ca^{2+}- and Mg^{2+}-free buffered salt solution such as TEN (40mM Tris-HCl, pH 7.5, 1mM EDTA, 150mM NaCl). The cells are scraped from the plate, pelleted, resuspended in Tris buffer and subjected to three freeze/thaw cycles to lyse cell membranes (1,2). This procedure is more time-consuming than a direct detergent cell lysis protocol, especially when the number of samples is large.

Luciferase lysis reagent is incompatible with CAT and beta-galactosidase assays

Promega's Luciferase Assay System includes a convenient Cell Culture Lysis Reagent (CCLR) which has been optimized for luciferase assays. Cells are washed with PBS and then incubated with the lysis

reagent for 15 minutes. The cell lysates are scraped from the dish, transferred to a microcentrifuge tube, spun briefly and used in the enzyme assay. Luciferase activity is more stable in cell lysates prepared in CCLR than in cell lysates prepared by the standard freeze/thaw method using Tris buffer (data not shown).

Unfortunately, CCLR is not optimal for beta-galactosidase and CAT enzyme assays. Addition of CCLR to the beta-galactosidase assay reagents produces turbidity and light scattering, rendering the spectrophotometric assay unreliable (data not shown). Cell lysates prepared with CCLR inhibit CAT activity 8- to 10-fold. This is shown in Figure 1, which compares the CAT activity measured when equivalent amounts of purified CAT enzyme were added to cell lysates prepared either with CCLR or with Tris buffer using the freeze/thaw method.

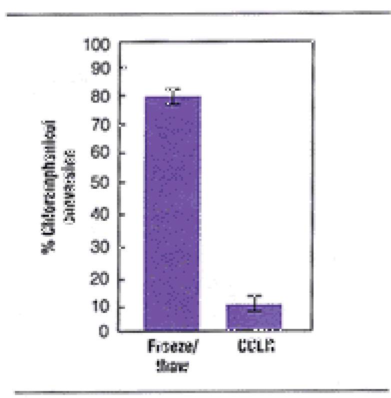

Figure 1. Luciferase Cell Culture Lysis Reagent inhibits CAT activity. Lysates were prepared from Balb/3T3 cells using either the standard freeze/thaw procedure or the Cell Culture Lysis Reagent (CCLR) supplied in the Luciferase Assay System. 40µl of cell extract was incubated at 37°C for 5 hours in a total volume of 100µl. Each reaction contained 0.1µCi of ^{14}C-chloramphenicol, 20µg of n-butyryl CoA and 0.01u of purified CAT enzyme. The reaction product, n-butyrylated chloramphenicol, was quantitated with a liquid scintillation counter following extraction with mixed xylenes.

Comparison of Reporter Lysis Buffer to standard lysis and assay reagents

A buffer compatible with luciferase, CAT and beta-galactosidase enzyme assays was produced by reformulating the lysis reagent with Bicine buffer and Tween® detergents. Figure 2 compares the enzyme activities measured in cell lysates prepared with this new Reporter Lysis Buffer or with the standard lysate method for each enzyme. In this study, replicate dishes of Balb/3T3 cells were transfected using Transfectam® Reagent and 5µg of pCAT®-Control DNA, or co-transfected with 5µg of pSV-beta-Galactosidase DNA plus 5µg of pGL2-Control DNA (Promega CAT, beta-galactosidase and luciferase reporter vectors, respectively). After 48 hours, transfected cells were harvested as indicated, either by the freeze/thaw method, with CCLR or with Reporter Lysis Buffer. Equal volume cell lysates were prepared for each comparison. The cell lysates used for CAT activity determinations were first heated at 60°C for 10 minutes to inactivate endogenous deacetylase activity (3). (Cell lysates to be assayed for both CAT and luciferase were aliquotted before heating, as luciferase activity is not stable to 60°C.) Beta-galactosidase activity was assayed spectrophotometrically (5), luciferase activity was measured using a luminometer and the standard conditions described in reference 6, and CAT activity was assayed using ^{14}C-chloramphenicol and the xylene extraction method (4).

Figure 2 shows that significantly higher beta-galactosidase activity was obtained in cell lysates prepared with Reporter Lysis Buffer than with the standard freeze/thaw protocol. Luciferase activities also were slightly higher when Reporter Lysis Buffer was used in place of CCLR. CAT enzyme activity was equivalent in lysates prepared with Reporter Lysis Buffer and the traditional freeze/thaw method.

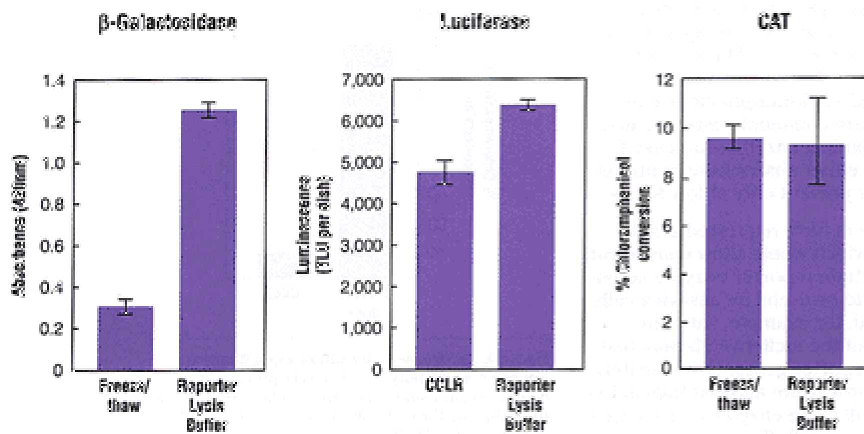

Figure 2. Reporter Lysis Buffer gives equivalent or better activities of beta-galactosidase, luciferase and CAT. Balb/3T3 cells were plated at 1.5 x 10^5 per 60mm dish and transfected the following day using either pCAT-Control DNA, or co-transfected with pSV-beta-galactosidase and pGL2-Control DNA. Cells were transfected using Transfectam Reagent and standard transfection techniques and were harvested 48 hours post-transfection. Cells were washed twice with PBS, lysed using either Reporter Lysis Buffer, Cell Culture Lysis Reagent (CCLR) or the freeze/thaw technique with Tris buffer, and brought to a final volume of 500µl.

Beta-Galactosidase Assay: 100µl of each cell extract was assayed with 150µl of 2X Assay Buffer (Beta-Galactosidase Enzyme Assay System) in a 300µl final volume. Samples were incubated for 30 minutes at 37°C, 500µl of 1M sodium carbonate was added, and the absorbance was read at 420nm. Data are presented as absorbance units per 60mm dish.

Luciferase Assay: 20µl of each extract was added to 100µl of Luciferase Assay Reagent (Luciferase Assay System). Emitted light was quantitated in a Turner Luminometer using a 2-second delay followed by a 10-second integration of light output. Data are expressed in light units per 60mm dish.

CAT Assay: 40µl of each cell extract was assayed in a total volume of 100µl containing 0.1µCi of chloramphenicol and 20µg of n-butyryl CoA, at 37°C for 2 hours. The product, n-butyrylated chloramphenicol, was quantitated with a liquid scintillation counter following extraction with mixed xylenes.

Summary

In summary, the new Reporter Lysis Buffer is compatible with luciferase, beta-galactosidase and CAT reporter enzyme assays. This new lysis protocol is faster and easier to use than the traditional freeze/thaw methods used for CAT and beta-galactosidase reporter assays. For luciferase assays, Reporter Lysis Buffer gives activities and stabilities roughly comparable to CCLR.

References

1. Gorman, C. *et al.* (1982) *Mol. Cell. Biol.* **2**, 1044.
2. Ausubel, F.M. (ed.) *et al.* (1992) *Current Protocols in Molecular Biology*, Chapter 9, "Introduction of DNA into Mammalian Cells," John Wiley and Sons, New York.
3. Ginot, F. *et al.* (1989) *Eur. J. Biochem.* **180**, 289.
4. *CAT Enzyme Assay System Technical Bulletin, #TB084,* Promega Corporation.
5. *Beta-Galactosidase Enzyme Assay System Technical Bulletin, #TB097,* Promega Corporation.
6. *Luciferase Assay System Technical Bulletin, #TB101,* Promega Corporation.

Ordering Information

Reporter Lysis Buffer is available as an individual product, and also is included at no extra charge in the newly configured CAT, luciferase and beta-galactosidase assay systems listed here. The Luciferase Assay System also is available with Cell Culture Lysis Reagent (CCLR) for those customers who wish to continue their studies using this buffer.

Product	**Size**	**Cat.#**
Luciferase Assay System with Reporter Lysis Buffer	100 Assays	E4030
Luciferase Assay System with CCLR	100 Assays	E1500
Luciferase Assay 10-Pack	1,000 Assays	E1501
CAT Enzyme Assay System	50 Assays	E1000
Beta-Galactosidase Enzyme Assay System	65 Assays	E2000
Reporter Lysis Buffer, 5X	30ml	E3971
Luciferase Cell Culture Lysis Reagent, 5X	30ml	E1531
Transfectam® Reagent for the Transfection of Eukaryotic Cells	0.5mg (25 transfections)	E1232
	1mg (50 transfections)	E1231

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