

NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection System

Catalog no. N10561, N10562

Table 1. Contents and storage information.

Material	N10561	N10562	Storage*	Stability
NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit (Cat. no. N10561, N10562) $\leq -20^{\circ}\text{C}$ Components				
Assay buffer (Component A)	1 each	3 \times 1 each	• $\leq -20^{\circ}\text{C}$	When stored as directed this kit is stable for 1 year.
Substrate dilution buffer (Component B)	1 each	3 \times 1 each		
NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit (Cat. no. N10561, N10562) 2–6°C Components				
Lysis buffer (Component A)	70 mL	210 mL	• 2–6°C • Protect from light • DO NOT FREEZE	When stored as directed this kit is stable for 1 year.
Galacton-Plus® substrate (Component B)	200 μL	600 μL		
Accelerator (Component C)	25 mL	75 mL		
Number of assays: Sufficient material is supplied for 200 (Cat. no. N10561) or 600 (Cat. no. N10562) microplate assays based on the protocol below.				

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 NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection System allows 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 NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection System, with detection of 1 fg to 20 ng or 10 fg to 20 ng of purified luciferase or β -galactosidase, respectively.

Description of the System

The NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection System incorporates the luminescent luciferin and Galacton-Plus® substrates for the detection of luciferase and β -galactosidase, respectively. Cell lysate is mixed with assay buffer for the luciferase reaction and the luciferase signal is measured immediately after the injection of substrate dilution buffer, 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 which raises the pH and provides the Sapphire-II™ luminescence enhancer to increase light intensity. Light emission from the β -galactosidase reaction exhibits glow kinetics with a half-life of 180 minutes. Residual light from the luciferase reaction is minimal, due to rapid kinetic signal decay and quenching by accelerator. Generally, only very high luciferase concentrations (ng levels of enzyme) interfere with detection of β -galactosidase. A longer delay after the addition of accelerator prior to measurement results in decreased residual luciferase signal when extremely high levels are present. However, it is important to maintain consistent timing of addition of substrate dilution buffer and measurement of the β -galactosidase signal after adding accelerator.

Applications

The NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection System has been very widely used for the following applications:

- Reporter quantitation/transfection normalization from transiently transfected mammalian cell lines^{3–9}, and transfected primary cells^{10–13}
- Viral studies, including gene expression regulation,¹⁴ retroviral infection,¹⁵ and viral replication¹⁶
- Readout for both siRNA gene expression inhibition¹⁷ and miRNA regulation in conjunction with Applied Biosystems pMIR-REPORT™ vectors¹⁸
- Mammalian two-hybrid analysis¹⁹
- RNA splicing assay²⁰
- Protein-protein interaction analysis²¹
- Assaying extracts from yeast cells containing a novel reporter fusion construct for measuring protein translation²²

Before Starting

Materials Required but Not Provided

- Reporter-construct transfected mammalian cells
- Phosphate buffered saline (PBS)
- Sterile water
- 96- or 384-well microplates (solid white or clear-bottom white)
- Microplate luminometer
- *Optional:* DTT
- *Optional:* β -galactosidase (Sigma Cat. no. G-5635) and luciferase (Sigma Cat. no. L-9506)

Caution

The assay buffer and substrate dilution buffer are irritating to eyes, respiratory system, and skin. If swallowed, seek medical advice immediately and show this container or label.

The Galacton-Plus[®] substrate is highly flammable and irritating to eyes. Vapors may cause drowsiness and dizziness. Keep container tightly closed. Keep away from sources of ignition. No smoking. Avoid contact with eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

The accelerator is irritating to eyes and skin. In case of contact with eyes, rinse immediately

with plenty of water and seek medical advice. Wear suitable protective clothing and eye/face protection.

General Guidelines

- 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. Adjust the amount of cell extract used to ensure the assay is within the linear range. The linear range of detection varies according to the cell type and on the reporter enzyme expression level.
- The NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection System is suitable for use with luminometers with automatic injectors. If only a single injector is available, rinse injector thoroughly between injection of substrate dilution buffer and accelerator. Manual addition of accelerator may be performed if luminescence intensities are measured at the same interval after adding accelerator.
- 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. Optimize the number of cells used per well 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 Technical Support for additional questions.
- The lysis buffer (100 mM potassium phosphate, pH 7.8, 0.2% Triton® X-100) included with the kit may be substituted with alternative lysis buffers, as long as all samples are prepared with the same lysis buffer. However, reducing agents interfere with the Galacton-Plus® substrate, causing high background and rapid signal decay. Carefully evaluate alternative lysis buffers 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 performing the assay due to a detrimental effect on luciferase. If high endogenous β -galactosidase activity necessitates heat inactivation, perform assays for luciferase and β -galactosidase on individual aliquots of the cell extract.

Controls

Recommended positive and negative controls are listed below.

β -Galactosidase Positive Control

Prepare stock enzyme by reconstituting lyophilized β -galactosidase (Sigma Cat. no. 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 Positive Control

Prepare stock enzyme by reconstituting lyophilized luciferase (Sigma Cat. no. 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, assay uninduced cells as a negative control for total assay background.

Prepare Buffers

Assay buffer: Reconstitute the lyophilized contents from each vial in 5 mL of sterile water. After reconstitution, store assay buffer at 4°C for 1 week, or aliquot and store at -20°C.

Substrate dilution buffer: Reconstitute the lyophilized contents from each vial in 22 mL of sterile water. After reconstitution, store substrate dilution buffer at 4°C for 1 week, or aliquot and store at -20°C.

Experimental Protocols

Prepare Extracts From Cultured Cells

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

1.1 *Optional:* Add DTT (to 0.5 mM) to the required volume of lysis buffer.

Note: DTT at a final concentration of 0.5 mM may help preserve the luciferase activity, but it may have adverse effects on the background and kinetics of the β -galactosidase assay. If extended light emission is critical (injector is not used for accelerator addition), avoid any reducing agents. If a lysis buffer containing excess DTT is used, add hydrogen peroxide to the accelerator at a final concentration of 10 mM (add 1 μ L of 30% H_2O_2 per 1 mL accelerator) to prevent rapid decay of signal half-life.

1.2 Rinse cell cultures twice with PBS.

1.3 Add lysis buffer (100 mM potassium phosphate, pH 7.8, 0.2% Triton® X-100) to cover the cells using 250 μ L solution per 60 mm plate.

1.4 Detach cells from plate with a cell scraper.

1.5 Transfer the cell lysate to a microcentrifuge tube and centrifuge for 2 minutes to pellet the debris.

1.6 Transfer extract (supernatant) to a fresh tube. Use the extract immediately for *Chemiluminescent Detection Protocol* or store at -80°C.

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). Heat inactivation of endogenous β -galactosidase activity is not effective with this protocol.

2.1 *Optional:* Add DTT (to 0.5 mM) to the required volume of lysis buffer.

Note: DTT may help to preserve luciferase activity, but it may have adverse effects on the background and kinetics of the β -galactosidase assay. See **Note** above.

- 2.2 Remove cell culture medium. Rinse cell cultures once with PBS (optional).
- 2.3 Add 10 μ L of lysis buffer to each well and incubate for 10 minutes.
- 2.4 Continue with the *Chemiluminescent Detection Protocol* (below) omitting step 3.3.

Chemiluminescent Detection Protocol

Perform all assays in triplicate at room temperature.

- 3.1 Equilibrate assay buffer and substrate dilution buffer to room temperature.
- 3.2 Dilute Galacton-Plus[®] substrate 1:100 in substrate dilution buffer. Prepare only enough for single day's use (100 μ L/well).
- 3.3 Transfer 2–10 μ L of extracts to microplate wells.

Note: The amount of extract used may vary depending on the level of expression and the instrumentation used. Use lysis buffer to adjust each sample to the same volume, if necessary.
- 3.4 Add 25 μ L of assay buffer to each well.
- 3.5 Within 10 minutes, inject 100 μ L/well of substrate dilution buffer (containing Galacton-Plus[®] substrate from step 3.2). After a 1–2 seconds delay, read the luciferase signal for 0.1–1 second/well.

Note: Signal intensities for both reporter enzyme reactions are time dependent. We do not recommend using manual addition of substrate dilution buffer, since the luciferase reaction reaches maximum light intensity within seconds and decays rapidly. Manual addition of accelerator solution is possible, as long as accelerator is added in the same consistent time frame as the substrate dilution buffer addition, such that the incubation time with substrate dilution buffer is identical for each sample. Instruments with automatic injection eliminate this concern. Longer or shorter measurements and delay times may be utilized but use the same timing when reading the β -galactosidase signal after addition of accelerator.
- 3.6 Incubate for 30–60 minutes at room temperature.
- 3.7 Inject 100 μ L accelerator. After a 1–2 seconds delay, read the β -galactosidase signal for 0.1–1 second/well (see **Note** above).

References

1. BioTechniques 21, 520 (1996); 2. In Bioluminescence and Chemiluminescence: Molecular Reporting with Photons (Hastings, JW, Kricka, LJ and Stanley, PE, eds), John Wiley, Chichester, England p 451-457 (1997); 3. J Biol Chem 272, 5283 (1997); 4. Biochem J 370, 719 (2003); 5. Oncogene 22, 5554 (2003); 6. Breast Cancer Research 6, R546-R555 (DOI 10.1186/bcr913) (2004); 7. Oncogene 24, 3427 (2005); 8. Mol Cancer Ther 6, 37 (2007); 9. Cancer Res 67, 6737 (2007); 10. Development 122, 2415 (1996); 11. J Biol Chem 272, 15817 (1997); 12. Circulation 106, 57 (2002); 13. Mol Pharmacol 63, 119 (2003); 14. J Biol Chem 282, 37146 (2007); 15. Mol Cell Biol 23, 831 (2003); 16. J Virology 77, 9885 (2003); 17. Proc Natl Acad Sci USA 99, 6047(2002); 18. J Biol Chem 283, 9836 (2008); 19. Biochemical Pharmacology 69, 867 (2005); 20. Hum Mol Genet 12, 1337 (2003); 21. Nuc Acids Res 33, 1 (2005); 22. Nucl Acids Res 32, 6605 (2004).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
N10561	NovaBright™ β-galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit *200 assays*	1 kit
N10562	NovaBright™ β-galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit *600 assays*	1 kit

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