

NovaBright™ β -galactosidase Enzyme Reporter Gene Chemiluminescent Detection System for Yeast or Mammalian Cells

Catalog no. N10563, N10564, N10565, N10566

Table 1. Contents and storage information.

| Material | N10563 | N10564 | N10565 | N10566 | Storage* | Stability |
|--|--------|--------|--------|--------|--|--|
| Galacton-Star® substrate concentrate (Component A) | 1 mL | 4.2 mL | 1 mL | 4.2 mL | <ul style="list-style-type: none">• 2–6°C• Protect from light• DO NOT FREEZE | When stored as directed this kit is stable for 1 year. |
| Assay buffer *for mammalian cells* (Component B) | 20 mL | 100 mL | -- | -- | | |
| Assay buffer *for yeast cells* (Component B) | -- | -- | 20 mL | 100 mL | | |

Number of assays: Sufficient material is supplied for 200 (Cat. no. N10563, N10565) or 1,000 (Cat. no. N10564, N10566) microplate assays based on the protocol below.

Introduction

The NovaBright™ β -galactosidase Enzyme Reporter Gene Chemiluminescent Detection System is designed for the rapid, simple, and sensitive detection of β -galactosidase reporter enzyme directly in microwell cultures of mammalian or yeast cells. The assay system incorporates the Galacton-Star® substrate with a luminescence enhancer to generate glow light emission kinetics. A single reagent, which provides cell lysis components and luminescent substrate and enhancer, is added directly to cells in culture medium. Light emission typically reaches maximum intensity within 60–90 minutes and remains constant for 45–90 minutes or longer (depending upon assay temperature, see Figure 1). Light signal is measured in a luminometer, without the need for automatic injection. The simple assay format and glow light emission achieved with the NovaBright™ β -galactosidase Enzyme Reporter Gene Chemiluminescent Detection System reagents provide an ideal assay system for automated high-throughput screening applications, enabling simple processing and measurement of multiple microplates. The dynamic range of the assay spans five orders of magnitude, from picogram to nanogram levels, enabling detection of a wide range of reporter enzyme concentration in cells.

The NovaBright™ β -galactosidase Enzyme Reporter Gene Chemiluminescent Detection System provides a chemiluminescent assay format that is sensitive, simple to use, and designed specifically for optimal performance in automated, high-throughput screening applications. The protocol is adaptable for use in multiple microplate formats with mammalian or yeast cells.

Applications

The bacterial β -galactosidase gene is widely used as a reporter enzyme for the study of gene regulation, for identification of protein-protein interactions and in assays for cell fusion.

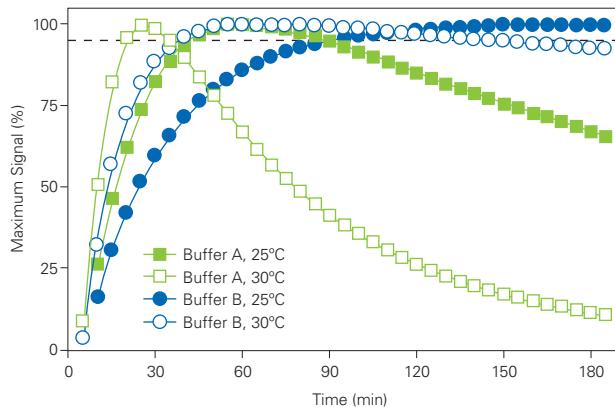


Figure 1. NovaBright™ β -galactosidase Enzyme Reporter Gene Chemiluminescent Detection assays were performed with purified β -galactosidase (500 pg) with assay buffer for mammalian cells (Buffer A) or assay buffer for yeast cells (Buffer B). Repeated measurements were made in the Applied Biosystems TR717™ microplate luminometer at room temperature (25°C) or 30°C. Data is presented as percentage of maximum signal, and dotted line indicates 95% of maximum signal.

Chemiluminescent 1,2-dioxetane substrates for β -galactosidase, including Galacton-Plus® and Galacton-Star® substrates, provide highly sensitive enzyme detection¹⁻⁴ and have been utilized extensively in reporter assays in mammalian cell and tissue extracts, and in a combined assay for luciferase and β -galactosidase activities^{5,6} (NovaBright™ β -galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection System, Invitrogen Cat. no. N10561 and N10562).

The NovaBright™ β -galactosidase Enzyme Reporter Gene Chemiluminescent Detection System is used widely for traditional reporter gene assays in:

- Transiently and stably-transfected mammalian cells^{7,8} including assays for studying viral infectivity and function⁹⁻¹²
- Yeast cells,¹³⁻¹⁵ including quantitative yeast two hybrid¹⁷⁻¹⁹ and one hybrid analysis²⁰
- Fish cells²¹
- Bacterial cells²²
- β -galactosidase complementation assays used for intracellular monitoring of protein-protein interactions,²³ protein translocation,²⁴ cell fusion,²⁵ and receptor dimerization/activation,^{26,27} including for high-throughput compound screening for receptor activation²⁸

Before Starting

Materials Required but Not Provided

- β -galactosidase reporter expressing mammalian or yeast cells
- 96- or 384-well tissue culture-treated microplates (solid white or clear-bottom white)
- Microplate luminometer
- *Optional:* β -galactosidase (Sigma Cat. no. G-5635)

Caution

Component A is highly flammable and irritating to eyes. Vapors may cause drowsiness and dizziness. Keep the container tightly closed and 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.

General Guidelines

Assay Plates

The use of white, tissue culture-treated microplates is recommended for optimal assay sensitivity. Clear-bottom white plates can be used to allow microscopic examination of cultures. White backing sheets may be applied to the plate bottom prior to signal measurement, if desired. The absolute signal will be higher (approximately 2-fold), since the white backing reflects light toward the photomultiplier tube detector and eliminates light absorption by the black plate platform, but relative signal levels are unaffected. Black plates can be used but are not recommended, since they cause reduced light yield due to absorbance of the luminescent signal.

When using 384-well plates, reduce the culture and assay reagent volume to 25 μ L (or as desired, maintaining a 1:1 ratio of culture:reagent volume without overfilling the wells).

Culture Medium

Mammalian cell culture medium containing phenol red indicator can be used without affecting assay sensitivity. The inclusion of phenol red can result in a reduction of signal due to absorbance of some of the emitted light, but the signal/noise is not affected. The type of culture medium and presence/absence of serum may contribute to some slight variability in light signal and assay kinetics. Initially perform a kinetic analysis of the particular cell line/culture medium system to determine optimum signal measurement time. It is not necessary to measure light signal at the time of peak light emission, as long as all samples are measured in a consistent time frame.

Endogenous β -galactosidase Activity

Chemiluminescent β -galactosidase reporter assays may be conducted in mammalian cells that have endogenous β -galactosidase activity. The reaction is performed at a pH which is more favorable for bacterial enzyme activity, which has a higher pH optimum than the endogenous mammalian enzyme. Assay the level of endogenous enzyme in non-transfected cells to determine the assay background signal.

Assay Temperature

Assays are ideally performed at 26–28°C. If the incubation temperature is significantly different, the kinetics of light emission vary, since the rate of an enzyme reaction is dependent on temperature (see Figure 1).

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 varies according to cell type and on the reporter enzyme expression level. 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.

Controls

The recommended positive and negative controls are described 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.

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.

| | |
|------------------------|--|
| Amount of Cells | For mammalian cell cultures, a typical cell density is 10,000–50,000 cells/well in 100 μ L for 96-well plates or 1,000–10,000 cells/well in 25 μ L for 384-well plates. For yeast cells, a typical cell density is 10,000–75,000 cells/well in 100 μ L for 96-well plates or 5,000–40,000 cells/well in 25 μ L for 384-well plates. |
|------------------------|--|

Experimental Protocol

β -Galactosidase Detection Protocol

Perform all assays in duplicate or triplicate. See guidelines above for preparing positive and negative controls.

Assay buffer for mammalian cells is optimized for mammalian cell cultures, and has been used with transfected adherent and non-adherent cell lines, including NIH/3T3, CHO-K1, and K562. Assay buffer for yeast cells is optimized for yeast cells, and is also been shown to work with mammalian cells. The alternative buffer formulations result in differing light emission kinetics. For mammalian cells, the assay buffer for mammalian cells provides faster kinetics, which may be advantageous at lower incubation temperatures. The choice of reagents depends on the particular assay requirements and desired kinetic performance.

- 1.1 Prepare diluted substrate fresh each time. Dilute the substrate 1:25 with assay buffer (i.e., mix 40 μ L substrate with 960 μ L assay buffer, 100 μ L/well required) to make reaction assay buffer.
- 1.2 Add 100 μ L/well of reaction assay buffer to a 96-well plate containing 100 μ L/well of cells in culture medium.
- 1.3 Incubate the samples at 26–28°C for approximately 60–90 minutes or until constant light emission is reached.
- 1.4 Place the plate in a luminometer and measure for 0.1–1 second/well.

References

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

| Cat. no. | Product Name | Unit Size |
|-------------------------|--|-----------|
| N10563 | NovaBright™ β-galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit *for mammalian cells* *200 assays* | 1 kit |
| N10564 | NovaBright™ β-galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit *for mammalian cells* *1000 assays* | 1 kit |
| N10565 | NovaBright™ β-galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit *for yeast cells* *200 assays* | 1 kit |
| N10566 | NovaBright™ β-galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit *for yeast cells* *1000 assays* | 1 kit |
| Related Products | | |
| 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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