Nano-Glo® Live Cell Assay System

Instructions for Use of Products N2011, N2012 and N2013.

Promega

Quick Protocol

The Nano-Glo® Live Cell Reagent is a nonlytic detection reagent that can be used to monitor luminescence from NanoLuc® luciferase or NanoBiT® assays in living cells. The detection reagent contains the substrate furimazine, which is cell permeable, and a proprietary agent for reducing autoluminescence and decreasing assay background.

If performing NanoBiT® assays, consult the *NanoBiT® Protein:Protein Interaction System Technical Manual* #TM461 for detailed protocols.

Preparation of the Nano-Glo® Live Cell Reagent

1. Equilibrate Nano-Glo® LCS Dilution Buffer to ambient temperature if using for the first time.
2. Remove the Nano-Glo® Live Cell Substrate from storage, and mix.
3. If the Nano-Glo® Live Cell Substrate has collected in the cap or on the sides of the tube, briefly spin tubes containing substrate in a microcentrifuge.
4. Prepare the desired amount of reconstituted Nano-Glo® Live Cell Reagent by combining 1 volume of Nano-Glo® Live Cell Substrate with 19 volumes of Nano-Glo® LCS Dilution Buffer (a 20-fold dilution). For example, if the experiment requires 20ml of reagent, add 1ml of substrate to 19ml of dilution buffer.

Notes:

1. Store the Nano-Glo® Live Cell Substrate at –20°C. Thaw the Nano-Glo® LCS Dilution Buffer upon first use and store at room temperature.
2. The Nano-Glo® Live Cell Reagent should be made fresh for each experiment. We do not recommend long-term storage of this solution at any temperature.
3. Once reconstituted, the reagent will lose 10% activity in approximately 3 hours at 20°C. At 4°C, the reconstituted reagent will lose 10% activity in approximately 7 hours.

Measuring Luminescence in 96-Well Plates

1. Equilibrate Nano-Glo® Live Cell Reagent to ambient temperature if chilled.
2. Aspirate medium from cells in 96-well plates and replace with 100µl of buffered cell culture medium, such as Opti-MEM® I Reduced Serum Medium (Life Technologies Cat.# 11058), containing 0–10% (v/v) fetal bovine serum (FBS).

   Note: We recommend a buffered cell culture medium (e.g., containing HEPES) to maintain physiological pH while measuring luminescence outside of the CO_2 incubator.

Continued

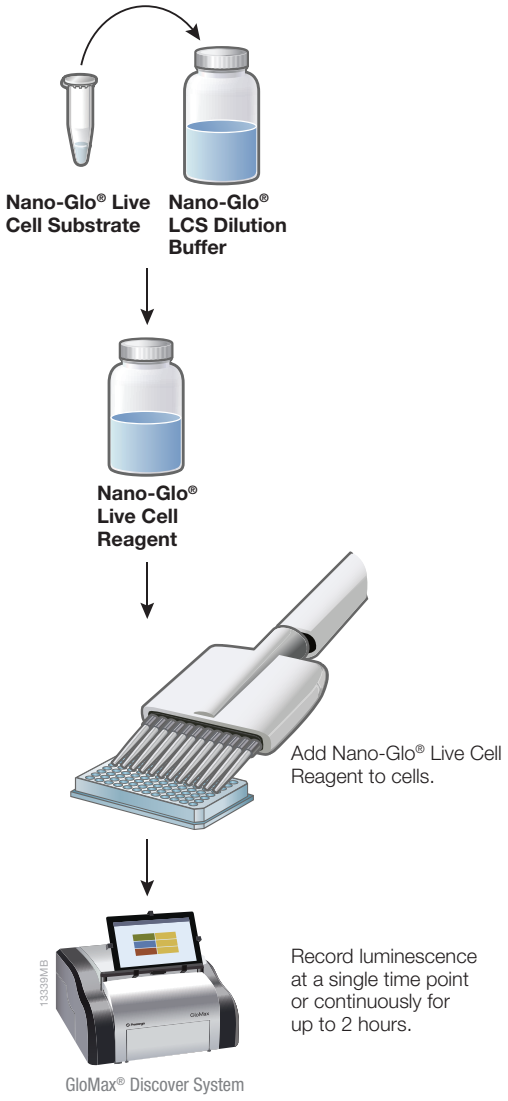

Figure 1. Flow diagram showing the preparation and use of the Nano-Glo® Live Cell Reagent.

Measuring Luminescence in 96-Well Plates (continued)

3. Add 25µl of Nano-Glo® Live Cell Reagent to each well, and gently mix the plate by hand or with an orbital shaker (e.g., 15 seconds at 300–500 rpm). **Note:** Cells remain intact.
4. Measure luminescence immediately after adding the Nano-Glo® Live Cell Reagent for a single time point. Alternatively, measure continuously over 2 hours for multiple time points. Use an integration time of 0.25–2 seconds.

   Note: Signal intensity and stability will vary, depending on experimental conditions. At high levels of luminescence, the rate of signal decay can increase due to increased enzymatic turnover and loss of furimazine substrate. At lower levels of luminescence, the rate of signal decay will be determined primarily by furimazine instability (Figure 2).

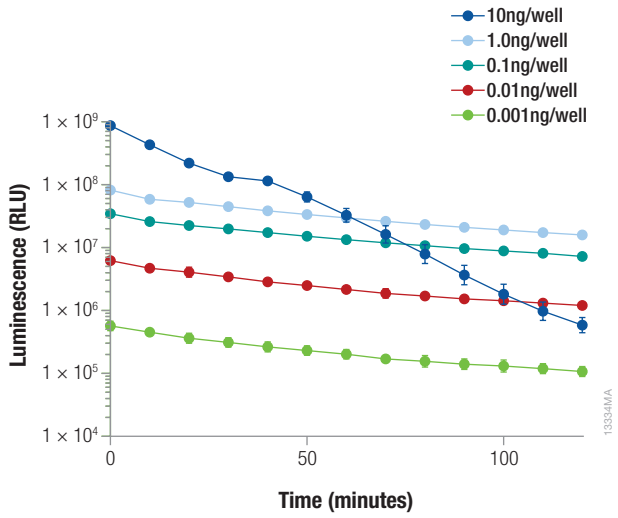

Figure 2. Monitoring signal stability of the Nano-Glo® Live Cell Reagent in HEK293 cells. HEK293 cells were transfected with varying amounts of the pNL1.1.CMV [*Nluc*/CMV] Vector encoding NanoLuc® luciferase. After 24 hours, cell culture medium was exchanged for Opti-MEM® I Reduced Serum Medium, and Nano-Glo® Live Cell Reagent was added. Luminescence was measured during the indicated time points at 37°C. The total amount of DNA transfected per well (50ng) was kept constant using Transfection Carrier DNA (Cat.# E4881). n = 3; error bars represent standard deviation.

Additional protocol information is in Technical Manual #TM461, available online at: **www.promega.com**

