Bio-Glo-NL™ Luciferase Assay System

Instructions for Use of Products J3081, J3082 and J3083.

Quick Protocol

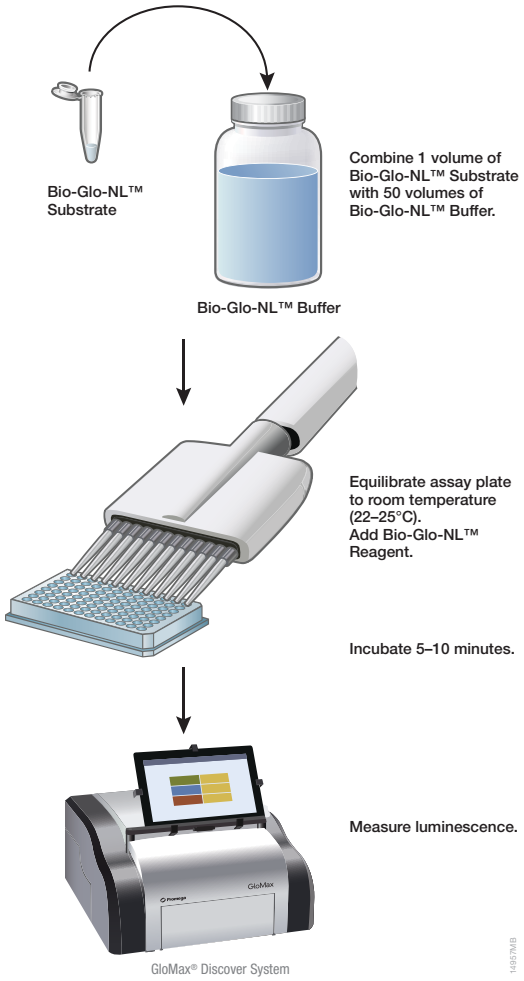
This Quick Protocol provides instructions for the Bio-Glo-NL™ Luciferase Assay System designed for use with the quantitative mechanism-of-action (MOA)-based Reporter Bioassays using the NanoLuc® luciferase reporter. For detailed instructions including plate setup, please refer to the *TIM-3 Bioassay Technical Manual* #TM590, available at: **www.promega.com/protocols/**

Preparing Bio-Glo-NL™ Luciferase Assay Reagent

Store Bio-Glo-NL™ Luciferase Assay Buffer and Bio-Glo-NL™ Luciferase Assay Substrate at –30°C to –10°C upon receiving. Once thawed, the buffer may be stored at 4°C for 1 year or at room temperature for 3 months with minimal change in performance. We recommend storing the substrate at –30°C to –10°C at all times. The substrate remains in the liquid phase in this temperature range.

We recommend preparing Bio-Glo-NL™ Reagent immediately before each use. Do not store and use the reconstituted reagent. Once reconstituted, the reagent will lose 10% activity in approximately 8 hours at room temperature.

1. Remove the Bio-Glo-NL™ Luciferase Assay Buffer from –30°C to –10°C storage and equilibrate to room temperature (do not exceed 25°C).
2. Remove the Bio-Glo-NL™ Luciferase Assay Substrate from –30°C to –10°C storage. Briefly centrifuge the tubes and then mix by pipetting.
3. Prepare the desired amount of reconstituted Bio-Glo-NL™ Luciferase Assay Reagent by combining one volume of substrate with 50 volumes of buffer. For example, if the experiment requires 10ml of reagent, add 200µl of substrate to 10ml of buffer. 10ml of reagent is sufficient for 120 wells (two assay plates, using the inner 60 wells of each plate).



Adding Bio-Glo-NL™ Luciferase Assay Reagent

1. Remove assay plates from the 37°C incubator after the bioassay incubation period and equilibrate to room temperature for 10–15 minutes.
2. Using a multichannel pipette, add a volume of Bio-Glo-NL™ Reagent equal to the volume of cells only or cells/test sample mixtures to each assay well. Avoid creating any bubbles.

   Note: Bio-Glo-NL™ Reagent should be at room temperature before adding to the assay plates.
3. Incubate at room temperature for 5–10 minutes.
4. Measure luminescence using a luminometer or multimode plate-reader.

Note: Bio-Glo-NL™ Reagent is compatible with most plate-reading luminometers, with an integration time of 0.5–1 second/well. Relative luminescence unit (RLU) readings will vary with the sensitivity and settings of each instrument. The use of different instruments will affect the magnitude of raw RLUs and might affect the assay window for test samples.

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