Bio-Glo-NB™ VLP Luciferase Assay System

Quick Protocol

Instructions for Use of Products **JB1201, JB1202 and JB1203.**

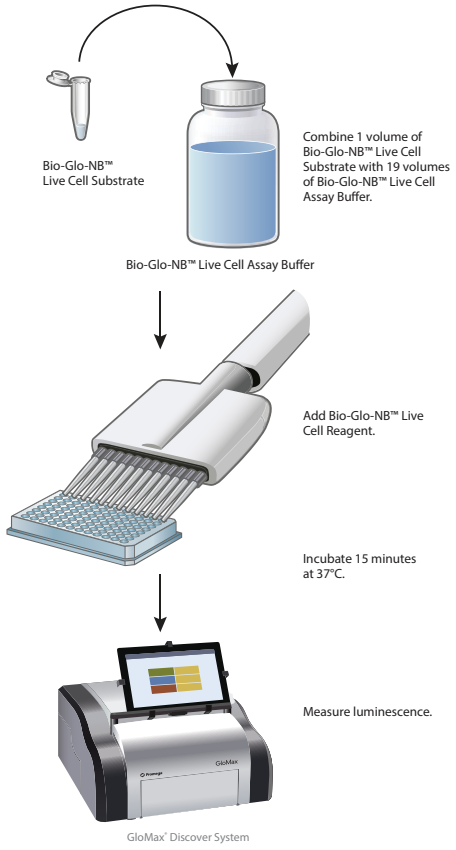
This Quick Protocol provides instructions for the Bio-Glo-NB™ VLP Luciferase Assay System designed for use with the HiBiT-tagged pseudovirus-like particle (HiBiT-PsVLP) Bioassays. For detailed instructions, including plate setup, please refer to a specific HiBiT-PsVLP Bioassay protocol.

Adding Bio-Glo-NB™ DrkBiT Peptide to Target Cells

Store Bio-Glo-NB™ DrkBiT Peptide at –30°C to –10°C upon receipt. Once thawed, Bio-Glo-NB™ DrkBiT Peptide should be used immediately. Do not store or refreeze Bio-Glo-NB™ DrkBiT Peptide after thawing.

1. Thaw one vial of Bio-Glo-NB™ DrkBiT Peptide at room temperature (do not exceed 25°C).
2. Prepare cell plating medium by adding Bio-Glo-NB™ DrkBiT Peptide to assay buffer according to your specific HiBiT-PsVLP Bioassay protocol.

   Note: Bio-Glo-NB™ DrkBiT Peptide must remain present throughout the assay to inhibit extracellular NanoBiT® complementation. Once added, do not remove medium containing Bio-Glo-NB™ DrkBiT Peptide.
3. Prepare and add LgBiT-expressing Target Cells to cell plating medium as directed by your specific HiBiT-PsVLP Bioassay protocol.



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Preparing Bio-Glo-NB™ Live Cell Reagent

Store Bio-Glo-NB™ Live Cell Assay Substrate and Buffer at –30°C to –10°C upon receipt. Once thawed, store the buffer at 4°C for 1 year or at room temperature for 3 months with minimal change in performance. The Bio-Glo-NB™ Live Cell Assay Substrate remains liquid at –30°C to –10°C. Prepare the Bio-Glo-NB™ Live Cell Reagent fresh for each experiment. **Do not** store or reuse the prepared reagent. 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.

1. Equilibrate Bio-Glo-NB™ Live Cell Luciferase Assay Buffer to room temperature if using for the first time.
2. Remove the Bio-Glo-NB™ Live Cell Substrate from storage and mix. If the Bio-Glo-NB™ Live Cell Substrate has collected in the cap or on the sides of the tube, briefly centrifuge substrate tubes.
3. Prepare the desired amount of reconstituted Bio-Glo-NB™ Live Cell Reagent by combining 1 volume of Bio-Glo-NB™ Live Cell Substrate with 19 volumes of Bio-Glo-NB™ Live Cell Buffer (a 20-fold dilution). For example, if the experiment requires 20ml of reagent, add 1ml of substrate to 19ml of buffer.

Adding Bio-Glo-NB™ Live Cell Reagent

1. Using a multichannel pipette, add a volume of room temperature Bio-Glo-NB™ Live Cell Reagent equal to the volume of medium in each assay well. For example, if the assay wells contain 100µl of medium, add 100µl of Bio-Glo-NB™ Live Cell Reagent to each well.
2. Gently mix the plate by hand or with an orbital shaker (e.g., 15 seconds at 300–500rpm).
3. Incubate at 37°C in a CO_2 incubator for 15 minutes.
4. Measure luminescence in a GloMax® Discover System (Cat.# GM3000) or a plate reader with glow-type luminescence reading capabilities. The luminescence intensity will decay gradually over the course of 2 hours.

Additional protocol information is available at: **www.promega.com**

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