Quick Protocol

Bio-Glo-NB™ Lytic Luciferase Assay System

Instructions for Use of Products **JB1101, JB1102 and JB1103**

This Quick Protocol describes use of the Bio-Glo-NB™ Lytic Luciferase Assay System with HiBiT Target Cell Killing (TCK) cells and primary macrophages in ADCP Bioassays. For detailed instructions, including plate setup, please enquire: **techserv@promega.com**

Preparing Bio-Glo-NB™ Lytic Reagent

Store Bio-Glo-NB™ Lytic Luciferase Assay Substrate, Bio-Glo-NB™ TCK LgBiT Protein and Bio-Glo-NB™ Lytic Luciferase Assay Buffer at –30°C to –10°C upon receipt. The Bio-Glo-NB™ Lytic Luciferase Assay Substrate and Bio-Glo-NB™ TCK LgBiT Protein remain as liquids and do not freeze.

We recommend preparing Bio-Glo-NB™ Lytic Reagent immediately before use. Equilibrate the Bio-Glo-NB™ Lytic Luciferase Assay Buffer to room temperature (do not exceed 25°C) before reconstituting the reagent. **Do not** store or reuse the reconstituted reagent. Once reconstituted, the reagent will lose ~15% activity over 8 hours and ~60% activity over 24 hours at room temperature.

1. Remove the Bio-Glo-NB™ Lytic Luciferase Assay Buffer from storage and equilibrate to room temperature (do not exceed 25°C).
2. Remove the Bio-Glo-NB™ Lytic Luciferase Assay Substrate from storage. Briefly centrifuge the tube and then mix by pipetting.
3. Remove the Bio-Glo-NB™ TCK LgBiT Protein from storage. Briefly centrifuge the tube and then mix by pipetting.
4. Transfer the desired amount of room temperature Bio-Glo-NB™ Lytic Luciferase Assay Buffer to a 15ml or 50ml centrifuge tube.
5. Add Bio-Glo-NB™ TCK LgBiT Protein (1:100 dilution) and Bio-Glo-NB™ Lytic Luciferase Assay Substrate (1:50 dilution) to the Bio-Glo-NB™ Lytic Luciferase Assay Buffer. For example, if the experiment requires 10ml of reagent, add 100µl of LgBiT Protein and 200µl of substrate to 10ml of buffer.

Adding Bio-Glo-NB™ Lytic Reagent

1. Remove assay plates from the incubator after the incubation period and equilibrate to room temperature for 10–15 minutes.
2. Using a multichannel pipette, add a volume of Bio-Glo-NB™ Lytic Reagent equal to the volume of cells only or cells/test sample mixtures to each assay well. Avoid creating bubbles.
3. Wait 10 minutes, then measure the luminescence in a GloMax® Discover System or a plate reader with glow-type luminescence reading capabilities. The luminescence intensity will decay gradually, with a signal half-life of 1–2.5 hours at room temperature.

   Note: Varying the Bio-Glo-NB™ Lytic Reagent incubation time will affect the raw relative light unit (RLU) values but should not significantly change the EC_{50} value and maximum fold induction.

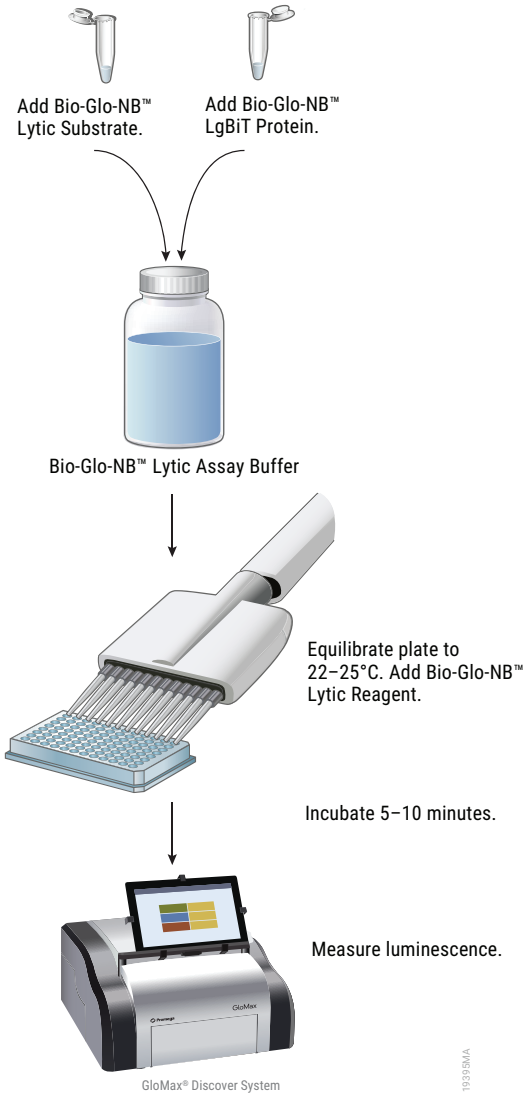

Figure 1. Bio-Glo-NB™ Lytic Luciferase Assay System protocol.