Bio-Glo™ Luciferase Assay System

Instructions for Use of Products G7940 and G7941.

Quick Protocol

This Quick Protocol provides instructions for the Bio-Glo™ Luciferase Assay System designed for use with the quantitative MOA-based Reporter Bioassays using firefly luciferase reporter. For detailed instructions including plate setup, please refer to the respective Fc Effector, T Cell Activation, Immune Checkpoint, Cytokines and Growth Factor Bioassay Technical Manual, available at: **www.promega.com/protocols/**

Preparing Bio-Glo™ Luciferase Assay Reagent

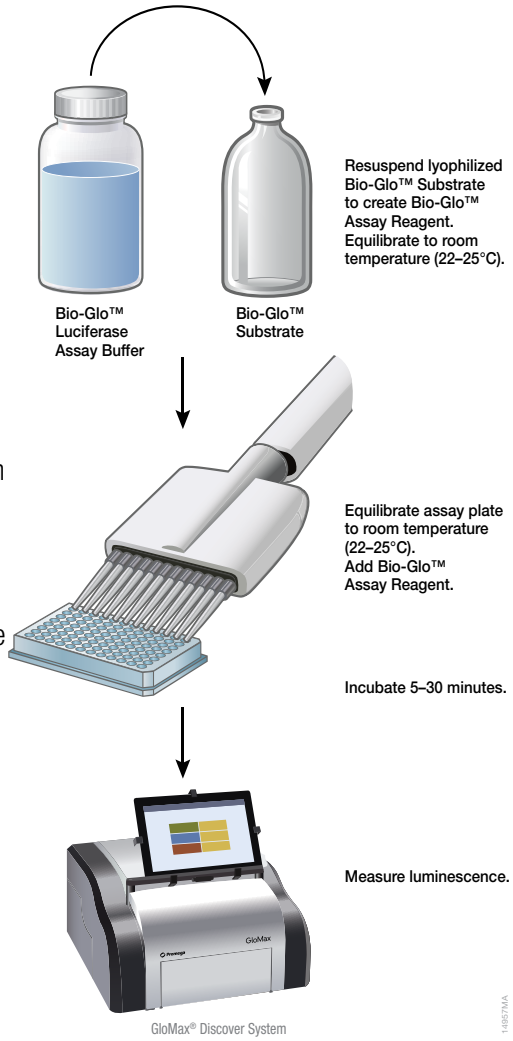
Store Bio-Glo™ Luciferase Assay Substrate and Bio-Glo™ Luciferase Assay Buffer at –20°C upon receiving. For optimal performance, use reconstituted Bio-Glo™ Reagent on the day of preparation. However, once reconstituted, Bio-Glo™ Reagent can be stored at –20°C for up to 6 weeks.

If you are using the 10ml size of Bio-Glo™ Luciferase Assay System (Cat.# G7941):

1. Thaw the Bio-Glo™ Luciferase Assay Buffer in a refrigerator overnight or in a room temperature water bath on the day of the assay.
2. Equilibrate the Bio-Glo™ Luciferase Assay Buffer to ambient temperature, protected from light.
3. Transfer the Bio-Glo™ Luciferase Assay Buffer into the amber bottle containing the Bio-Glo™ Luciferase Assay Substrate, and mix by inversion until the Substrate is thoroughly dissolved.
4. Equilibrate the reconstituted Bio-Glo™ Reagent to ambient temperature before adding to assay plates.

If you are using the 100ml size of Bio-Glo™ Luciferase Assay System (Cat.# G7940):

1. Thaw and reconstitute the Bio-Glo™ Reagent as above.
2. Dispense the reconstituted Bio-Glo™ Reagent into 10ml aliquots and store at –20°C for up to six weeks. Avoid repeated freeze-thaw cycles.
3. On the day of the assay, thaw the appropriate amount of reconstituted Bio-Glo™ Reagent in a room temperature water bath for 4–6 hours before use.



Note: Approximate stability of Bio-Glo™ Reagent after reconstitution is 18% loss of luminescence over 24 hours at ambient temperature.

Adding Bio-Glo™ Luciferase Assay Reagent

1. Remove assay plates from the 37°C incubator and equilibrate to ambient temperature (22–25°C) on the bench for 5–15 minutes.
2. Using a manual multichannel pipette, add a volume of Bio-Glo™ Reagent equal to the volume of cells only or cells/test sample mixtures to each assay well. Avoid creating any bubbles.

   Note: Bio-Glo™ Reagent should be at ambient temperature.
3. Incubate at ambient temperature for 5–30 minutes.
4. Measure luminescence using a luminometer or multimode plate-reader.

Note: Bio-Glo™ 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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