Getting Started
Sections 4 and 5 of Technical Manual #TM356 list the recommended supplies, equipment and reaction controls for the ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay.


Reagent Preparation
1. Thaw each assay component as follows:
   - Assay Buffer: 37°C water bath
   - GF-AFC Substrate: 37°C water bath (Vortex to ensure homogeneity.)
   - ONE-Glo™ Luciferase Assay Buffer: Room temperature
   - ONE-Glo™ Luciferase Assay Substrate: Room temperature
2. Transfer 10µl of the GF-AFC Substrate into 2.0ml of Assay Buffer for the 1 plate size. Transfer 100µl of the GF-AFC Substrate into 20ml of Assay Buffer for the 10 plate size. This mixture constitutes the 5X CellTiter-Fluor™ Cell Viability Assay Reagent.
   Note: See Technical Manual #TM356 for addition information on customizing the CellTiter-Fluor™ Reagent for various multiwell plates, volume added, and reagent storage information.
3. Transfer the contents of the bottle of ONE-Glo™ Buffer into the amber bottle containing ONE-Glo™ Substrate. Mix by swirling or inverting the contents until the substrate is thoroughly dissolved to create the ONE-Glo™ Reagent.
   Note: See Technical Manual #TM356 for ONE-Glo™ Reagent storage information.

ONE-Glo™ + Tox Assay Protocol
Example Assay Protocol for 96-Well Plate Format
1. Set up 96-well assay plates containing cells capable of expressing firefly luciferase in medium at the selected density.
2. Add test compounds and vehicle controls to appropriate wells for a final volume of 100µl per well.
3. Culture cells for the desired test exposure period and under conditions resulting in luciferase reporter expression.
   Note: When characterizing new compounds, use multiple exposure periods to assess the full effect on cellular health.
4. Add 20µl of 5X CellTiter-Fluor™ Reagent to all wells, and briefly mix by orbital shaking (300–500rpm for ~30 seconds).
5. Incubate for 30 minutes at 37°C.
   Note: Incubations longer than 30 minutes may improve assay sensitivity and dynamic range. However, do not incubate more than 3 hours.
7. Add 100µl of ONE-Glo™ Reagent to all wells.
   Note: The half-life of the ONE-Glo™ Reagent is generally greater than 45 minutes but may be influenced by medium formulation and solvents.
8. Incubate for 3 minutes at room temperature.

Protocol continued on the next page.
Example Assay Protocol for 384-Well Plate Format

1. Set up 384-well assay plates containing cells capable of expressing firefly luciferase in medium at the selected density.
2. Add test compounds and vehicle controls to appropriate wells for a final volume of 25µl per well.
3. Culture cells for the desired test exposure period and under conditions resulting in luciferase reporter expression.
   Note: When characterizing new compounds, use multiple exposure periods to assess the full effect on cellular health.
4. Add 5µl of 5X CellTiter-Fluor™ Reagent to all wells, and briefly mix by orbital shaking (1,000-1,200rpm for ~30 seconds).
5. Incubate for 30 minutes at 37°C.
   Note: Incubations longer than 30 minutes may improve assay sensitivity and dynamic range. However, do not incubate more than 3 hours.
6. Measure fluorescence at 380–400nmEx/505nmEm (viability).
7. Add 25µl of ONE-Glo™ Reagent to all wells.
   Note: The half-life of the ONE-Glo™ Reagent is generally greater than 45 minutes but may be influenced by medium formulation and solvents.
8. Incubate for 3 minutes at room temperature.

For additional protocol information including General Considerations, see Technical Manual #TM356, available online at: www.promega.com/protocols/