

ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay

Quick
PROTOCOL

INSTRUCTIONS FOR USE OF PRODUCTS E7110 AND E7120.

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.

For complete protocol information, see the *ONE-Glo™ + Tox Luciferase Reporter and Cell Viability Assay Technical Manual #TM356*, available at: www.promega.com/protocols/

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.
6. Measure fluorescence at 380–400nm_{Ex}/505nm_{Em} (viability).
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.
9. Measure luminescence (luciferase reporter gene expression).

Protocol continued on the next page.

ORDERING/TECHNICAL INFORMATION:

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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 380–400nm_{Ex}/505nm_{Em} (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.
9. Measure luminescence (luciferase reporter gene expression).

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

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