ApoLive-Glo™ Multiplex Assay

INSTRUCTIONS FOR USE OF PRODUCTS G6410 AND G6411.

Quick PROTOCOL

Reagent Preparation

1. Thaw each assay component as follows: Assay Buffer: 37°C water bath; GF-AFC Substrate: 37°C water bath; Caspase-Glo® 3/7 Buffer: Room temperature; Caspase-Glo® 3/7 Substrate: Room temperature
2. Transfer the contents of the GF-AFC Substrate into Assay Buffer.
   For 96-well plates, transfer 10µl of substrate into 2ml of Assay Buffer.
   For standard 384-well plates, transfer 10µl of substrate into 2.5ml of Assay Buffer.
   Mix the Assay Buffer containing substrates by vortexing the contents until the substrate is thoroughly dissolved. This mixture will be referred to as the Viability Reagent.
3. Transfer the contents of one Caspase-Glo® 3/7 Buffer bottle into one amber bottle containing Caspase-Glo® 3/7 Substrate. Mix by swirling or inverting the contents until the substrate is thoroughly dissolved to form the Caspase-Glo® 3/7 Reagent (~20 seconds).

   Note: See the Technical Manual #TM325 for information about storing reconsituted reagents.

Sample Assay Protocol 96-well Plate

1. Set up 96-well assay plates containing cells in medium at the selected density.

   Note: We recommend using <20,000 per well in a 96-well plate.
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.
4. Add 20µl of Viability 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 the following wavelength set: $400_{Ex}/505_{Em}$.
7. Add 100µl of Caspase-Glo® 3/7 Reagent to all wells, and briefly mix by orbital shaking (300–500rpm for ~30 seconds).
8. Incubate for 30 minutes at room temperature.

   Note: Incubation times longer than 30 minutes may improve assay sensitivity and dynamic range.
9. Measure luminescence.

See additional protocol information in Technical Manual #TM325, available online at **www.promega.com/tbs**

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ApoLive-Glo™Multiplex Assay

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Sample Assay Protocol 384-well Plate

1. Set up 384-well assay plates containing cells in medium at the desired density.

   Note: We recommend using <5,000 cells per well in a 384-well plate.
2. Add test compounds and vehicle controls to appropriate wells for a final volume of 20µl per well.
3. Culture cells for the desired test exposure period.
4. Add 5µl of Viability Reagent to all wells, and briefly mix by orbital shaking (1,300–1,500rpm 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 the following wavelengths: $400_{Ex}/505_{Em}$.
7. Add 25µl of Caspase-Glo® 3/7 Reagent to all wells, and briefly mix by orbital shaking (1,300–1,500rpm for ~30 seconds).
8. Incubate for 30 minutes at room temperature.

   Note: Incubation times longer than 30 minutes may improve assay sensitivity and dynamic range.
9. Measure luminescence.

See additional protocol information in Technical Manual #TB325, available online at **www.promega.com/tbs**

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