ApoTox-Glo™ Triplex Assay

INSTRUCTIONS FOR USE OF PRODUCTS G6320 AND G6321.

Quick PROTOCOL

Getting Started

Section 4 of Technical Manual #TM322 lists the recommended supplies, equipment and reaction controls for the ApoTox-Glo™ Triplex Assay.

For complete protocol information, see the *ApoTox-Glo™ Triplex Assay Technical Manual* #TM322, available at: **www.promega.com/tbs/**

Reagent Preparation

1. Thaw each assay component as follows:
   - Assay Buffer: 37°C water bath
   - GF-AFC Substrate: 37°C water bath
   - bis-AAF-R110 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 and bis-AAF-R110 Substrate into 2.0 or 2.5ml of Assay Buffer, depending on the plate format used. For 96-well plates, transfer 10µl of each substrate into 2ml of Assay Buffer. For standard 384-well plates, transfer 10µl of each substrate into 2.5ml of Assay Buffer. Mix the Assay Buffer containing substrates by vortexing the contents until the substrates are thoroughly dissolved. This mixture will be referred to as the Viability/Cytotoxicity Reagent.

   Note: See Technical Manual #TM322 for Viability/Cytotoxicity Reagent storage information.
3. Transfer the contents of the Caspase-Glo® 3/7 Buffer bottle into the 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 Technical Manual #TM322 for Caspase-Glo® 3/7 Reagent storage information.

ApoTox-Glo™ Triplex Assay Protocol

Example Assay Protocol for 96-Well Plate Format

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

   Note: We recommend using <20,000 cells 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.

   Note: When characterizing new compounds, it is important to use in multiple exposure periods to assess the full effect on cellular health.
4. Add 20µl of Viability/Cytotoxicity Reagent containing both GF-AFC Substrate and bis-AAF-R110 Substrate 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.

Protocol continued on the next page.

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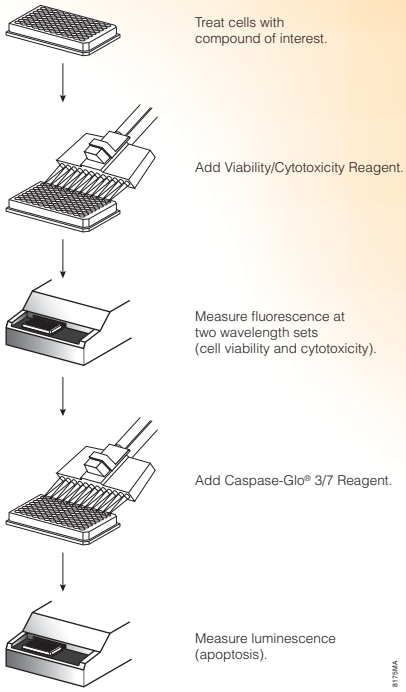
ApoTox-Glo™ Triplex Assay Protocol (continued)

6. Measure fluorescence at the following two wavelength sets:
   - $400_{Ex}/505_{Em}$ (Viability)
   - $485_{Ex}/520_{Em}$ (Cytotoxicity)
7. Add 100µl of Caspase-Glo® 3/7 Reagent to all wells, and briefly mix by orbital shaking (300–500rpm for ~30 seconds).

   Note: Incubation times longer than 30 minutes may improve assay sensitivity and dynamic range. See Note in the Before You Begin Section of Technical Manual #TM322 for more information.
8. Incubate for 30 minutes at room temperature.
9. Measure luminescence (caspase activation, a hallmark of apoptosis).



Example Assay Protocol for Standard 384-Well Plate Format

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.

   Note: When characterizing new compounds, it is important to use in multiple exposure periods to assess the full effect on cellular health.
4. Add 5µl of Viability/Cytotoxicity Reagent containing both GF-AFC Substrate and bis-AAF-R110 Substrate 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 two wavelength sets:
   - $400_{Ex}/505_{Em}$ (Viability)
   - $485_{Ex}/520_{Em}$ (Cytotoxicity)
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).

   Note: Incubation times longer than 30 minutes may improve assay sensitivity and dynamic range. See Note in the Before You Begin Section of Technical Manual #TM322 for more information.
8. Incubate for 30 minutes at room temperature.
9. Measure luminescence (caspase activation, a hallmark of apoptosis).

For additional protocol information including the Recommended Control Experiment and General Considerations, see Technical Manual #TM322, available online at: **www.promega.com/tbs/**

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