CellTiter-Glo® Luminescent Cell Viability Assay

Instructions for Use of Products G7570, G7571, G7572 and G7573

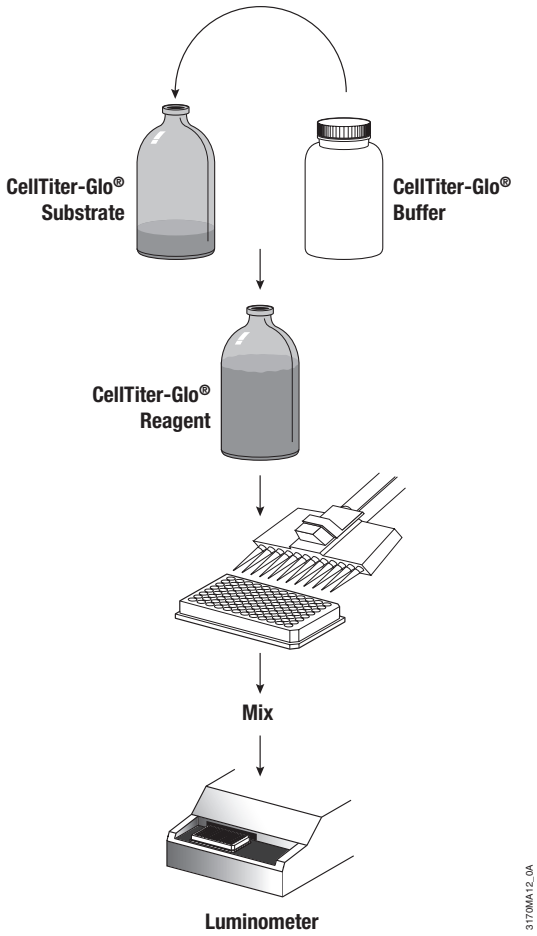
Quick Protocol

This Quick Protocol provides instructions for use of the CellTiter-Glo® Luminescent Cell Viability Assay, designed for use with multiwell formats, making the assay ideal for automated high-throughput screening applications. For detailed instructions, please refer to the *CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin* #TB288, available at: **www.promega.com/protocols/**

Reagent Preparation

Store the lyophilized CellTiter-Glo® Substrate and CellTiter-Glo® Buffer at –30°C to –10°C for long-term storage. For frequent use, the CellTiter-Glo® Buffer can be stored at room temperature for 48 hours without loss of activity. See product label for expiration date information. Reconstituted CellTiter-Glo® Reagent (Buffer plus Substrate) can be stored at room temperature for up to 8 hours with <10% loss of activity, at 4°C for 48 hours with ~5% loss of activity, at 4°C for 4 days with ~20% loss of activity or at –20°C for 21 weeks with ~3% loss of activity. The reagent is stable for up to ten freeze-thaw cycles, with less than 10% loss of activity.

1. Thaw the CellTiter-Glo® Buffer, and equilibrate to room temperature prior to use. For convenience, CellTiter-Glo® Buffer can be thawed and stored at room temperature for up to 48 hours prior to use.
2. Equilibrate the lyophilized CellTiter-Glo® Substrate to room temperature prior to use.
3. Transfer the appropriate volume (10ml for Cat.# G7570 and G7571; 100ml for Cat.# G7572 and G7573) of CellTiter-Glo® Buffer into the amber bottle containing CellTiter-Glo® Substrate to reconstitute the lyophilized enzyme/substrate mixture. This forms CellTiter-Glo® Reagent.
4. Mix by gently vortexing, swirling or inverting the contents to obtain a homogeneous solution. The CellTiter-Glo® Substrate should go into solution easily in less than 1 minute.



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Protocol for the Cell Viability Assay

We recommend that you perform a titration of your particular cells to determine the optimal number and ensure that you are working within the linear range of the CellTiter-Glo® Assay.

1. Prepare opaque-walled multiwell plates with mammalian cells in culture medium, 100µl per well for 96-well plates or 25µl per well for 384-well plates.

   Note: Multiwell plates must be compatible with the luminometer used.

2. Prepare control wells containing medium without cells to obtain a value for background luminescence.
3. Add the test compound to experimental wells and incubate according to culture protocol.
4. Equilibrate the plate and its contents at room temperature for approximately 30 minutes.
5. Add a volume of CellTiter-Glo® Reagent equal to the volume of cell culture medium present in each well (e.g., add 100µl of reagent to 100µl of medium containing cells for a 96-well plate or add 25µl of reagent to 25µl of medium containing cells for a 384-well plate).
6. Mix contents for 2 minutes on an orbital shaker to induce cell lysis.
7. Incubate the plate at room temperature for 10 minutes to stabilize the luminescent signal.

   Note: Variations in luminescent signal within standard plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

8. Record luminescence.

   Note: Instrument settings are determined by the manufacturer. An integration time of 0.25–1 second per well should serve as a guideline.