CellTiter-Glo® 2.0 Assay

Instructions for Use of Products G9241, G9242 and G9243

Quick Protocol

This Quick Protocol provides instructions for the CellTiter-Glo® 2.0 Assay designed for use with multiwell-plate formats, making the assay ideal for automated high-throughput screening applications.

The CellTiter-Glo® 2.0 Reagent is a ready-to-use reagent based on the original CellTiter-Glo® Luminescent Cell Viability Assay chemistry and eliminates the need to combine buffer with lyophilized substrate when preparing reagent. For detailed instructions, please refer to the *CellTiter-Glo® 2.0 Assay Technical Manual* #TM403, available at: **www.promega.com/protocols/**

Reagent Preparation

Store at –30°C to –10°C. For maximum light signal, we recommend long-term storage at less than –65°C. Functional performance (linearity, sensitivity and signal half-life) of the reagent is maintained upon storage at –30°C to –10°C through the expiration date, although light output will decline over time.

CellTiter-Glo® 2.0 Reagent can be thawed and stored at +2°C to +10°C for up to 2 months with >85% light output remaining when measuring 1µM ATP in a standard reaction. Do not refreeze the thawed reagent after extended storage above –10°C. Do not dispense CellTiter-Glo® 2.0 Reagent into aliquots due to the risk of ATP contamination.

CellTiter-Glo® 2.0 Reagent is light-sensitive and should be stored in an opaque container.

1. If frozen, thaw CellTiter-Glo® 2.0 Reagent at 4°C overnight.

   Notes:

   a. Reagent may also be thawed in a 22°C water bath.

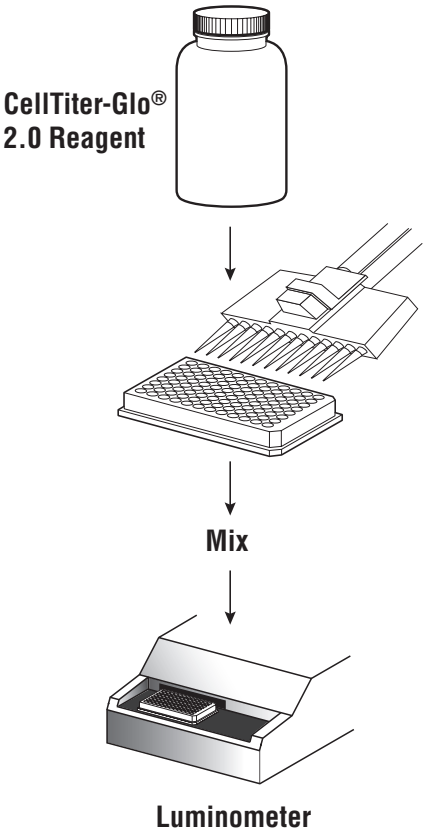
   b. Do not expose the reagent to temperatures above 25°C.

   c. If the CellTiter-Glo 2.0 Reagent is stored at –65°C, do not directly transfer it into a 22°C water bath to thaw. Instead, to avoid potentially cracking the bottle due to the rapid temperature change, leave the material on the bench top for 10–15 minutes and then place it in a 22°C water bath.

2. If not at room temperature, equilibrate the CellTiter-Glo® 2.0 Reagent to room temperature by placing the reagent in a 22°C water bath prior to use.

   Note: In a 22°C water bath, 100ml of the thawed reagent (4°C) requires approximately 30 minutes to equilibrate, and 500ml requires approximately 100 minutes to equilibrate.

3. Mix gently by inverting the contents to obtain a homogeneous solution.
   Note: Use caution when removing the seal of the CellTiter-Glo® 2.0 Reagent bottle to avoid introducing ATP contamination.



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Quick Protocol

Protocol for the Cell Viability Assay

1. Prepare opaque-walled multiwell plates with mammalian cells in culture medium. Volumes and cell number should be optimized for experimental conditions.

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

2. If desired, prepare control wells containing medium without cells to determine background luminescence.
3. Add test compound to experimental wells and incubate according to your culture protocol.
4. Equilibrate the plate and its contents to room temperature for approximately 30 minutes.
5. Add a volume of CellTiter-Glo® 2.0 Reagent equal to the volume of cell culture medium present in each well (e.g., for a 96-well plate, add 100µl of CellTiter-Glo® 2.0 Reagent to 100µl of medium containing cells).
6. Mix the contents for 2 minutes on an orbital shaker to induce cell lysis.
7. Allow the plate to incubate at room temperature for 10 minutes to stabilize the luminescent signal.
8. Record luminescence.

   Notes:

   a. Instrument settings depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.

   b. Uneven luminescent signal within plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

Sequential Multiplexing of CellTox™ Green Cytotoxicity Assay and CellTiter-Glo® 2.0 Assay

1. Completely thaw the CellTox™ Green Dye in a 37°C water bath. Mix the CellTox™ Green Dye using a vortex mixer to ensure homogeneity. A brief centrifugation may be necessary for complete recovery of the CellTox™ Green Dye. Although performance of CellTox™ Green Dye is optimal in black plates, white plates are optimal for sequential multiplexing formats that include a luminescent second measure.
2. Add the CellTox™ Green Dye at seeding or dosing so the final CellTox™ Green Dye concentration is 1X (stock = 1,000X) and incubate under desired conditions.
3. Equilibrate plate to room temperature.
4. Measure fluorescence intensity at 485–500nm$_{Ex}$/520–530nm$_{Em}$. Depending upon instrument manufacturer and optical detection configuration, replicate wells may benefit from 1 minute of shaking on an orbital shaker before measuring fluorescence.
5. Equilibrate the CellTiter-Glo® 2.0 Reagent to room temperature. After the final fluorescence measurement, add an equal volume of CellTiter-Glo® 2.0 Reagent to each well.
6. Place on an orbital shaker for 2 minutes and measure luminescence after 10 minutes.