CellTiter-Glo® 3D Cell Viability Assay

Instructions for Use of Products G9681, G9682 and G9683

Quick Protocol

This Quick Protocol provides instructions for the use of CellTiter-Glo® 3D Cell Viability Assay with multiwell plate formats, making the assay ideal for automated high-throughput screening applications.

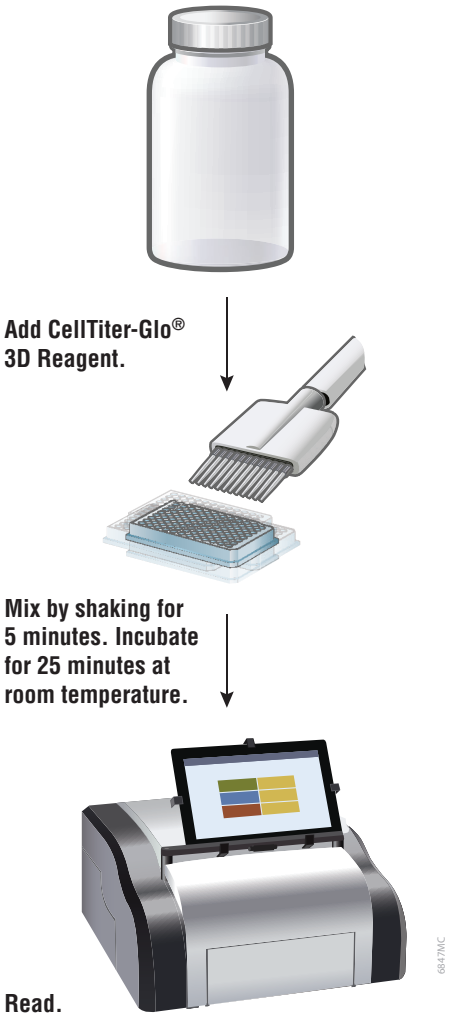
The CellTiter-Glo® 3D Cell Viability Assay is designed for use with 3D cell culture microtissues. It is formulated with more robust lytic capacity than CellTiter-Glo® 2.0 Reagent, which is designed for use with cell culture monolayers. For detailed instructions, please refer to the *CellTiter-Glo® 3D Cell Viability Assay Technical Manual* #TM412, available at: **www.promega.com/protocols/**

Reagent Preparation

The CellTiter-Glo® 3D Cell Viability Assay is shipped frozen. Store at –30°C to –10°C through the expiration date on the kit label. The CellTiter-Glo® 3D Reagent will retain >90% activity when stored at 4°C for 3.5 days or at room temperature (22°C–25°C) for 12 hours. The reagent can withstand three additional freeze-thaw cycles after the first thaw with no significant loss of activity. We do not recommend dispensing the CellTiter-Glo® 3D Reagent into aliquots due to the risk of ATP contamination.

1. Thaw the CellTiter-Glo® 3D Reagent at 4°C overnight.
   Do not thaw reagent by placing the frozen bottle directly into a waterbath as the bottle may break.
2. Equilibrate the CellTiter-Glo® 3D Reagent to room temperature by placing the reagent in a 22°C water bath for approximately 30 minutes.
3. Mix gently by inverting the contents to obtain a homogeneous solution.

   Note: Use caution when removing the seal of the CellTiter-Glo® 3D Reagent bottle to avoid introducing ATP contamination.



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Protocol for the Cell Viability Assay

1. Prepare opaque-walled multiwell plates with microtissues in culture medium. Sample volumes and microtissue properties (e.g., size, number, days in culture, etc.) should be optimized for experimental conditions. Multiwell plates must be compatible with the luminometer used.
2. Add test compound to experimental wells, and incubate according to your culture protocol. Be sure that the volume of the sample plus test compounds is low enough to allow addition of an equal volume of reagent, and subsequent mixing without well-to-well contamination.
3. Equilibrate the plate and its contents to room temperature (22–25°C) for approximately 30 minutes.
4. Add a volume of CellTiter-Glo® 3D 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® 3D Reagent to 100µl of medium containing cells).
5. Mix the contents vigorously for 5 minutes to induce cell lysis.

Note: Mixing is very important for effective extraction of ATP from 3D microtissues.

6. Incubate the plate at room temperature for an additional 25 minutes to stabilize the luminescent signal.
7. Record luminescence.

   Notes:

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

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

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