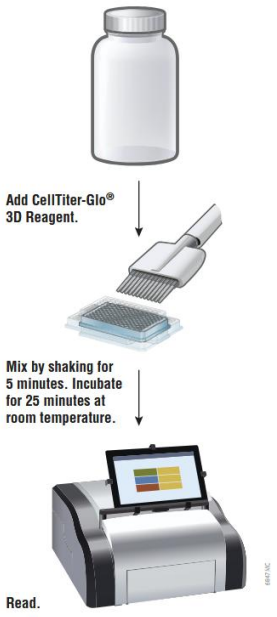
In Vitro Application

CellTiter- Glo® 3D Cell Viability Assay Protocol for 96-well and 384-well LifeGel Plates

CellTiter-Glo® is a luminescent assay that measures cell viability based on ATP levels, indicating metabolically active cells. It provides a sensitive, rapid, and easy-to-use method with a simple add-mix-read protocol.
CellTiter-Glo® 3D Cell Viability Assay (Promega) is specifically optimized for determining viability in 3D spheroids. The assay reagent penetrates large spheroids and has increased lytic capacity—allowing more accurate determination of viability compared to other assay methods.
CellTiter-Glo® 3D Cell Viability Assay measures ATP as an indicator of viability and generates a luminescent readout that is much more sensitive than colorimetric or fluorescence-based methods. The simple, 30-minute protocol and ready-to-use reagent allows for fast results.

The CellTiter-Glo® 3D Cell Viability Assay protocol (Promega).

Multiwell plates (96- or 384-well) compatible with fluorescent plate readers are prepared with cells and the compounds to be tested using standard methods.
CellTiter-Glo ® Reagent is added directly to each well, the plates are incubated at 37°C.



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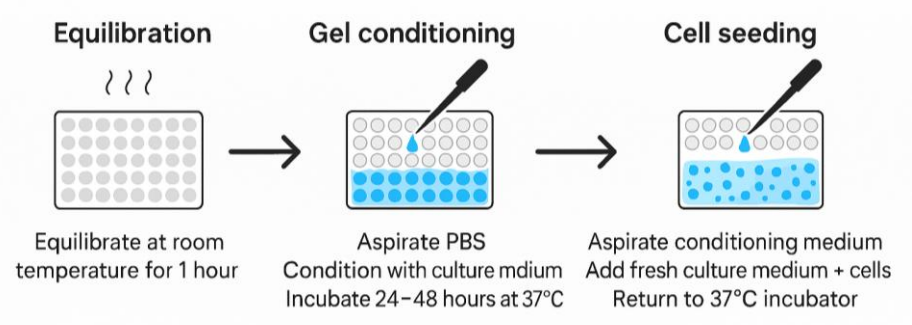
PROTOCOL:

Preparing 96- or 384-well LifeGel plates for CellTiter-Glo® 3D Cell Viability Assay

Materials required:

- 96- or 384-well opaque-walled LifeGel plate (Cat. No)
- Pipette/multichannel pipettor
- Complete culture medium

1. **Equilibration**
   Remove LifeGel plates from the refrigerator 24 or 48 hours before seeding. Allow them to equilibrate at room temperature for approximately 1 hour.
2. **Gel conditioning**
   Aspirate the PBS from each well. Add 150 µl of culture medium for 96-well plates or 60 µl for 384-well plates to condition the gel. Incubate the plates at 37°C in a humidified incubator.
3. **Cell seeding**
   After 24 or 48 hours of conditioning, remove the plates from the incubator.
   Aspirate the conditioning medium from the wells.
   Add 150 µl (96-well) or 60 µl (384-well) of fresh culture medium containing cell suspension to each well.
   Include appropriate control wells as needed*.
   Return the seeded LifeGel plates to the 37°C incubator for cell culture.



Tips:
Unlike CellTiter Blue reagent, CellTiter Glo 3D reagent can affect cell growth in neighbouring wells, so assaying wells on the same plate on different days is not recommended.

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PROTOCOL:

CellTiter-Glo® 3D Cell Viability Assay for 96- or 384-Well LifeGel Plates

1. **CellTiter-Glo® Reagent preparation**
   Thaw the CellTiter-Glo® Reagent and allow it to reach room temperature. *Important:* Protect the reagent from direct light during handling. Use caution when removing the seal of the CellTiter-Glo® 3D Reagent bottle to avoid introducing ATP contami-nation.
2. **Preparation of the culture plates**
   Remove assay plates from the 37°C incubator and allow them to equilibtare to room temperaturę.
3. **CellTiter-Glo® Reagent addition**
   The volume of CellTiter Glo 3D reagent added should be equal to the volume of culture medium above the LifeGel surface. To ensure enough space for addition and to conserve reagent use, partial removal of medium from wells using a multichannel pipette can be used.
   Remove 75µl of medium from the well and add 75µl of CellTiter-Glo® Reagent per well to 96-well LifeGel plates, and for 384-well LifeGel plates remove 30µl of medium from each well and add 30µl of CellTiter-Glo® Reagent.
4. **Incubation**
   Allow the plate to incubate in room temperature (22–25°C) for approximately 30 minutes.
5. **Record luminescence**
   Detection instrument settings depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.
   An uneven luminescent signal within plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates

***Recommendet controls:** No-Cell Control
Set up triplicate wells without cells to serve as the negative control.