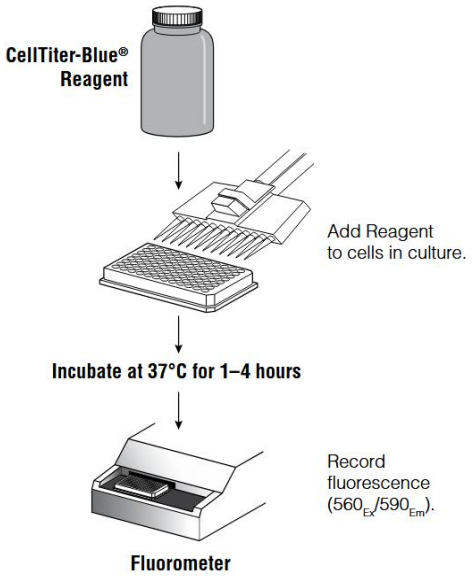
In Vitro Application

Cell-Titer Blue® Cell Viability Assay Protocol for 96-well and 384-well LifeGel Plates

The CellTiter-Blue® Cell Viability Assay (Promega) offers a simple, fluorescence-based method for assessing cell viability. It relies on the ability of metabolically active (live) cells to reduce the redox dye resazurin into the fluorescent compound resorufin. In contrast, nonviable cells quickly lose this metabolic function and fail to produce fluorescence. This homogeneous assay requires only the addition of a single reagent directly to cells cultured in serum-containing medium. Following a brief incubation period, fluorescence can be measured using a plate-reading fluorometer (preferred) or a spectrophotometer.

The CellTiter-Blue® 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-Blue ® Reagent is added directly to each well, the plates are incubated at 37°C to allow cells to convert resazurin to resorufin, and the fluorescent signal is measured.



Storage Conditions: Store the CellTiter-Blue ® Reagent frozen at –30°C to –10°C protected from light. Thaw CellTiter-Blue® Reagent completely and mix thoroughly before use. For frequent use, the product may be stored tightly capped at +2°C to +10°C or at ambient temperature (22–25°C) for 6–8 weeks. Protect from light. The product is stable for at least 10 freeze-thaw cycles.

REAL RESEARCH

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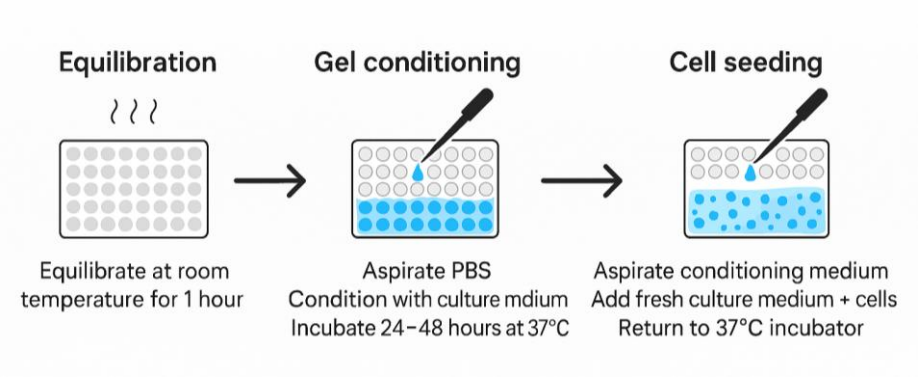
PROTOCOL:

Preparing 96- or 384-well LifeGel plates for CellTiter Blue® Assay

Materials required:

- 96- or 384-well LifeGel plate
- 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.



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PROTOCOL:

CellTiter-Blue® Cell Viability Assay for 96- or 384-Well LifeGel Plates

1. **CellTiter-Blue® Reagent preparation**
   Thaw the CellTiter-Blue® Reagent and allow it to reach room temperature. A 37°C water bath may be used to accelerate thawing.
   Important: Protect the reagent from direct light during handling.
2. **CellTiter-Blue® Reagent addition**
   Remove assay plates from the 37°C incubator.
   Add 25–30 µl of CellTiter-Blue® Reagent per well to 96-well LifeGel plates, or 7.5–15 µl of CellTiter-Blue® Reagent per well to 384-well LifeGel plates**.
3. **Mixing**
   Mix gently by shaking the plate for 10 seconds or by pipetting up and down to ensure even distribution.
4. **Incubation**
   4. Incubate the plates under standard cell culture conditions (37°C, 5% CO_2) for 1 to 4 hours, depending on signal strength required***.
5. **Fluorescent measurement**
   4. Measure fluorescence using a plate reader with excitation at 560 nm and emission at 590 nm.

***Recommendet controls:** No-Cell Control
Set up triplicate wells without cells to serve as the negative control to determine background fluorescence that may be present.
** **The amount of CellTiter-Blue® Reagent** required may vary depending on the cell type and cell density. Cells with higher metabolic activity or greater density will reduce the reagent more rapidly, generating a stronger fluorescent signal.
*** **Incubation time:**
Highly metabolic cells need shorter incubation time (1–2 hours). These cells rapidly reduce resazurin to resorufin, generating strong fluorescence signals quickly. Longer incubation may lead to signal saturation.
Moderately metabolic cells typically require a longer incubation time of 2–3 hours, while slow-metabolizing cells may need 3–4 hours to generate a detectable signal. Monitor signal development periodically to avoid over-incubation.
It's important to optimize reagent volume and incubation time for your specific cell model to ensure results fall within the linear range of detection and avoid over- or underestimation of viability.

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