PeptiMatrix™
Next generation peptide hydrogels

STANDARD OPERATING PROCEDURE

CELLTITER-GLO® 3D CELL VIABILITY ASSAY FOR CELLS IN PEPTIMATRIX HYDROGELS

1. BACKGROUND

The CellTiter-Glo® 3D Cell Viability Assay is a homogeneous ("add-mix-measure") method to determine the number of viable cells in 3D cell culture. The CellTiter-Glo® 3D Reagent comprises a proprietary thermostable luciferase (Ultra-Glo™ Recombinant Luciferase), which generates a stable quantifiable luminescent signal proportional to the ATP present, which is a marker for the presence of metabolically active cells. The CellTiter-Glo® 3D Reagent used in this assay has robust lytic capacity and is designed for use with 3D cell culture, and is therefore ideal for use with cells encapsulated within PeptiMatrix™ hydrogels. This assay is compatible with multiwell-plate formats, making it ideal for automated high-throughput screening using cell proliferation and cytotoxicity assays. The protocol is very quick and simple to carry out, where cell washing, removal of medium and multiple pipetting steps is not required.

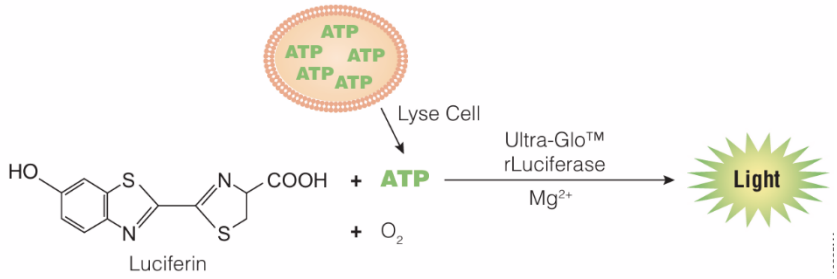

Figure 1 - CellTiter-Glo® 3D Cell Viability Assay principle.
Adapted from https://www.promega.com/-/media/files/resources/protocols/technical-manuals/101/celltiter-glo-3d-cell-viability-assay-protocol.pdf

2. RISK ASSESSMENT

Always follow your organisation's laboratory safety procedures.

Work inside an appropriate microbiology safety cabinet for your cell type. Refer to the **PeptiMatrix Safety Data Sheet (SDS)** for detailed safety, handling, storage, and first aid information relating to the hydrogel components.

If you are working with additional cell lines, media supplements, matrix additives, or other reagents consult the relevant SDS documents for those materials as well.

3. MATERIALS

- Class II microbiology safety cabinet (or appropriate class for your cell type)
- Cells encapsulated or seeded on PeptiMatrix hydrogels
- CellTiter-Glo® 3D Cell Viability Assay (Promega #G9681)
- Appropriate well size microplate e.g. white opaque walled 96 well plate for optimal luminescence signal with less well-to-well crosstalk (e.g. Greiner #655083 - 96 well white cell culture plate)
- P200 pipettes and filter tips
- Dulbecco's Phosphate Buffered Saline (DPBS)
- Foil
- Luminometer, CCD camera, or imaging device capable of reading luminescence in multiwell plates

Optional materials:

- 22°C water bath
- Foil
- Multichannel pipette
- Plate shaker
- ATP for use in generating a standard curve (e.g. Promega #P1132 - rATP 10mM 0.5 mL)

4. METHODS

4.1 Reagent preparation

This procedure can be scaled up or down depending on the number of wells you plan to prepare, and which well plate size you are using.

Note: Strict aseptic technique is essential to prevent ATP contamination of the CellTiter-Glo® 3D Reagent. Wear gloves and avoid contact with potentially contaminated surfaces and equipment. Clean gloves, lab surfaces and equipment with a 10% bleach solution, then pat dry with lab wipes. Use ATP-free pipette tips, and avoid inserting pipettors or pipette tips into the CellTiter-Glo® 3D Reagent bottle multiple times. Discard any unused, dispensed reagent; do not return it to the original bottle.

1. Remove CellTiter-Glo® 3D Reagent from ≤-20 °C and thaw at 4°C overnight.
    - **Do not** thaw reagent in water bath as the frozen bottle may break.

2. Equilibrate the plate with cells encapsulated in hydrogels and the CellTiter-Glo® 3D Reagent to **room temperature** (22–25°C) for 30 minutes (e.g. in 22°C water bath).
    - Temperature equilibration is vital as temperature gradients can cause uneven luminescence signals.
3. Mix CellTiter-Glo® 3D Reagent by gentle inversion to obtain a homogeneous solution.

4.2. CellTiter-Glo® 3D cell viability protocol

This procedure can be scaled up or down depending on the number of wells you plan to prepare, and which well plate size you are using.

For reference, the steps below describe the process for cells encapsulated in **100 µL hydrogel** per well plated into a **96-well plate**.

Note: Strict aseptic technique is essential to prevent ATP contamination of the CellTiter-Glo® 3D Reagent. Wear gloves and avoid contact with potentially contaminated surfaces and equipment. Clean gloves, lab surfaces and equipment with a 10% bleach solution, then pat dry with lab wipes. Use ATP-free pipette tips and avoid inserting pipettors or pipette tips into the CellTiter-Glo® 3D Reagent bottle multiple times. Discard any unused, dispensed reagent; do not return it to the original bottle.

1. Using a P200 pipette, aspirate the old media on top of the gel from the well, removing most of the media volume without disturbing the gel at the bottom.
2. Add volume of CellTiter-Glo® 3D Reagent equal to the volume of hydrogel per well e.g. 100 µL reagent to 100 µL cells in hydrogel.
3. Mix the contents vigorously for 5 minutes.
    - It is recommended to use a plate shaker for this step. Vigorous pipetting (e.g. pipetting sample 8-10 times) can be used instead but typically produces greater signal variability than shaking.
    - Mixing is very important for effective extraction of ATP from 3D microtissues.
4. Allow the plate to incubate at room temperature for an additional 25 minutes to stabilise luminescence signal.
    - It is recommended to cover plate in foil.
5. Record luminescence.
    - Detection instrument settings will depend on the manufacturer.
    - Use integration time of 0.25-1 second per well as a guideline.

5. DISPOSAL

Dispose of hydrogels containing cells, media, or matrix components according to your local guidelines for biological waste.

Dispose of CellTiter-Glo 3D Reagent according to your institute guidelines.

6. DOCUMENT HISTORY

Version	Date	Summary of Changes
1.0	**05 Feb 26**	First version of customer facing SOP, adapted from internal PeptiMatrix CTG procedures.