SOP TITLE:	VERSION:	
PM-PD-SOP3.2 CellTiter-Glo 3D assay for cell viability	1	
AUTHOR & RESEARCH TEAM:	STATUS:	
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SOP REVIEWED AND APPROVED BY VJE (MAY 9, 2024, 12:26 PM +0100)

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1. Background

The CellTiter-Glo® 3D Cell Viability Assay(a) is a homogeneous method to determine the number of viable cells in 3D cell culture based on quantitation of the ATP present, which is a marker for the presence of metabolically active cells.

2. Materials

- CellTiter-Glo® 3D Cell Viability Assay (Promega)
- Plate reader with luminescence probe
- Class II Microbiological Safety Cabinet
- Cells for staining in 96-well plate (preferably opaque walled with optical polymer base)
- P200 filter tips
- P200 pipette
- DPBS

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Product	Supplier	Cat No	Storage
DPBS	Gibco	14190-094	RT
CellTiter-Glo 3D	Promega	G9681	-20°C

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3. METHODS

Reagent preparation

1. Thaw the CellTiter-Glo 3D reagent at 4°C overnight.
2. Equilibrate the reagent to room temperature in a 22°C water bath.
3. Mix gently by inverting the contents to obtain homogenous solution.

Staining protocol

1. Add volume of CellTiter-Glo 3D reagent equal to the volume of hydrogel present in each well.
2. Mix contents vigorously to induce cell lysis (e.g., using a microplate shaker).
    1. **NOTE:** Mixing is very important for effective extraction of ATP from 3D microtissues.
3. Allow plate to incubate at room temperature for an additional 25 minutes to stabilize luminescence signal.
4. Record luminescence.
    1. **NOTE:** Detection instrument settings depend on the manufacturer. Use an integration time of 0.25–1 second per well as a guideline.
    2. An uneven luminescent signal within plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

Staining gels in a cell culture insert

The same basic procedure as above can be used, but you may need to first remove the gels from the cell culture insert and transfer them to a 96-well plate prior to staining.

4. Disposal

1. Dispose of cell culture medium, and any solutions that have been in contact with cells, by pouring into waste pot/ aspirating into vacuum trap containing Chemgene. Leave in Chemgene for at least one hour.
2. Dispose of any unneeded glass in appropriate glass bins.
3. Dispose of contaminated sharps in appropriate sharps bins.

User Initials Legend

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