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Cell Viability Protocol using CellTiter-Glo 3D

Methods and Protocols

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol provides a viability measurement for 3D culture.

Materials

ATP (Mw = 551.14 g/mol) (A7699-1G, Sigma-Aldrich)
spheroids
Incomplete DMEM/F-12 culture medium without FBS (1%L-Glutamine, 1% Penicillin/Streptomycin) (Gibco 21331-020)
CellTiter-Glo® 3D Cell Viability Assay (Promega G968A)
Aluminium
Shaker
Opaque 96-well plate for reading (Costar 96 Flat Bottom White Polystyrene)
Luminescence reader (Tecan Infinite M Plex)

Preparation of the CellTiter-Glo 3D Solution

1 ●
Thaw the CellTiter-Glo reagent in the fridge at 4°C the day before the experiment.

2 ●
Before use, let the kit reach room temperature for 30 minutes.

3 ●
Gently mix the solution.

Preparation of ATP Solutions at Different Concentrations for the Calibration Curve

4 Dilution of the ATP Solution

	ATP Concentration (µM)	0	0.5	1	2	3
	ATP at 10 µM (µL)	0	10	20	40	60
	DMEM without FBS (µL)	200	190	180	160	140

5 ●
Transfer the ATP solutions into the opaque 96-well plate for luminescence

Preparation of Spheroids in the Opaque Plate

6 ●
Transfer the spheroids from the incubation plate to the opaque 96-well plate for reading.

7 ●
Place the plate on the 28-magnet

plate (Plate Holding) and aspirate the medium.

8 ● Add 50 µL of DMEM without FBS per well.

Addition of CellTiter-Glo and Incubation of the Opaque Plate

9 ● Add 50 µL of CellTiter-Glo per well (into wells containing 50 µL of DMEM without FBS).

10 ● Cover the plate with aluminum foil to protect the samples from light, as CellTiter-Glo is light-sensitive.

11 ● Incubate the plate at room temperature for 25 minutes.

12 ● During this incubation period, shake the plate on a vortex for 5 minutes at 2 rpm.

Luminescence Reading

13 ● Set up the device:
○ Shaking mode: Orbital shaking
○ Shaking time: 3 s
○ Attenuation: None
○ Integration time: 1000 ms
○ Settle time: 0 ms

○
Temperature:
22°C

14 ●
Place the plate without the lid on
the reader platform and position the plate.

15 ●
Start the reading and save the Excel
file.