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Cell viability using Calcein

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 measures the viability of cells within a spheroid after dissociation.

Materials

Spheroids
DPBS
Accumax (SCR006 sigma-aldrich)
24-well plate cell-repellent
for culture (Greiner Bio-one 662970)
Calcein AM-blue (c1429 Invitrogen)
agitator incubator
(Incu-shaker)
Flow cytometer (Fortessa)

Spheroid dissociation

1 -Place spheroids in a 24-well cell repellent plate, pooling 8 spheroids in a well.

2 -Remove medium and add 500µl/well of DPBS

3 Remove DPBS and add 500µl/well of accumax

4 Flush to break up spheroids

5 Incubate plate at 37°C in incu-shaker at 200rpm for 1h30, flushing regularly (45' +45' for hearts/crowns).

6 Recover and place in 2ml tubes

Calcein AM Blue staining

7 - Centrifuge 300g 5min - discard supernatant

8 - Add 500µl /tube of DPBS - suspend cells - centrifuge again - discard supernatant

9 - Add 500µl /tube of Calcein 7µg/ml

10 - Incubate for 15 min in the dark

11 Centrifuge - add 300µL/tube of DPBS

12 - Run on flow cytometer