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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

