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# Standard Protocol for Spheroid Formation in the 96-Well Plate by centrifugation

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 enables spheroid production by centrifugation.

## Materials

KPC A219 Cells
iMEF Cells
KPC A219 and iMEF are cultured separately in T75 flask in complete DMEM/F12 in the incubator (37°C, 5% CO2). They have two passages in a week.
Complete DMEM/F-12 Culture Medium (10% FBS, 1% L-Glutamine, 1% Penicillin/Streptomycin) (Gibco 21331-020)
DPBS (Gibco 14190-086)
Trypsin (Gibco 25300-054)
TGFβ Solution (Transforming Growth Factor) (0.01 mg/ml) (PEPROTECH 100-21)
Medium Aspirator
Tube Holder
Malassez/Thomas Hemocytometer
Blue Trypan
Micropipettes
20µL and 200µL (STAR LAB)
Pipetboy
T75 Culture Flask (Corning 430641U) / T25 Culture Flask (Thermo Fisher Scientific 156367)
96-Well Transparent Plate with Flat Bottom (Greiner Bio-One 655970) / 96-Well Transparent Plate with Round Bottom (Greiner Bio-One 650970)
15ml Centrifuge Tubes (Fisher Scientific 431885) / 50ml Centrifuge Tubes (Fisher Scientific 431176)

## Day -1 before cell passage : Addition of TGF Beta and Nanoshuttles

1 For the T75 iMEF flask, add 75 µL of TGFβ to achieve 50 ng/mL of TGFβ in the culture medium.
For the T25 iMEF flask, add 25 µL of TGFβ to achieve 50 ng/mL of TGFβ in the culture medium.

2 Incubate the flasks in the incubator (37°C, 5% CO2).

## Day 0: Co-culture and Spheroid Formation

3 **Cell Passage and Preparation of Cell Suspensions at $2\times10^6$ Cells/mL Concentration**

3.1 Warm the DMEM culture medium, DPBS, and trypsin in a water bath.

3.2 Aspirate the medium from the flasks.
Add 5 mL of DPBS to the T75 flask and 3 mL of DPBS to the T25 flask to rinse the cells.
Aspirate the DPBS.

3.3 Add 2 mL of trypsin to the T75 flask and 1 mL of trypsin to the T25 flask.
Incubate KPC A219 cells for 6 minutes and iMEF cells for 5 minutes in the incubator (37°C, 5% CO2).
Check if the cells detach properly from the flask walls.
Add 10 mL of DMEM to the T75 flask and 5 mL of DMEM to the T25 flask to inhibit the trypsin reaction.

3.4 Take 20 µL of cell suspension and mix it with 20 µL of Blue Trypan.
Distribute the mixed solution on the Malassez/Thomas cell counter, then count the cells.

3.5 Centrifuge the cell suspensions for 5 minutes at 300g in a centrifuge at room temperature.

3.6 Calculate the volume of DMEM needed to dilute or concentrate the cell suspensions to a concentration of $2\times10^6$ cells/mL:
Volume of DMEM to add = Total number of cells / $2\times10^6$
Aspirate the supernatant and add the calculated volume of DMEM to achieve a cell suspension at $2\times10^6$ cells/mL.
Homogenize the cell suspension by pipetting.

## Preparation of KPC and iMEF Mixing Solution

4 Calculate the volume of KPC suspension, iMEF suspension, and DMEM culture medium needed to form spheroids:
1 spheroid = 10,000 KPC + 20,000 iMEF
Mix the KPC suspension, iMEF suspension, and DMEM after the calculations.

## Dispensing the Mixed Solution into the 96-Well Plate

5 Dispense 150 µL of the mixed solution into each well of the 96-well plate round bottom.

## Plate centrifugation

6 Change the rotor adapted to plate
Equilibrate with another plate with the same volume
Centrifuge at room temperature 4 minutes, at 300G
Rotate the plate in 180°
Centrifuge again at room temperature 4 minutes, at 300G

## Cell Incubation

7 Incubate the cells in the incubator (37°C, 5% CO2)
Perform treatment 4 days after