Mar 28, 2025

Standard Protocol for Magnetic Spheroid Formation

Methods and Protocols

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4z772vo5/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.3byl4z772vo5/v1

External link: https://doi.org/10.3390/mps8040075

Protocol Citation: Marine Simonneau 2025. Standard Protocol for Magnetic Spheroid Formation . **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4z772vo5/v1

Manuscript citation:
Perier M, Wang L, Simonneau M, Ngo-Reymond J, Guillermet-Guibert J, Lafond M, Lafon C (2025) Formation and Characterization of Two Magnetic Three-Dimensional Spheroid Models of Murine Pancreatic Adenocarcinoma. Methods and Protocols 8(4). doi: 10.3390/mps8040075

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

Created: March 28, 2025

Last Modified: March 28, 2025

Protocol Integer ID: 125617

Keywords: pancreatic adenocarcinoma spheroid model, magnetic spheroid formation, standard protocol for magnetic spheroid formation, pancreatic cancer cell, spheroid formation, using magnetic aggregation, magnetic aggregation, spheroid, fibroblast

Abstract

Our study introduces three-dimensional (3D)
pancreatic adenocarcinoma spheroid models using magnetic aggregation of
pancreatic cancer cells and immortalized fibroblasts

Materials

- KPC A219 Cells
- iMEF Cells
- Nanoshuttles (Greiner Bio-One 657846)
- 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)
- 96-Magnet Plate (Greiner Bio-One 130188)
- 24-Magnet Plate
- Medium Aspirator
- Tube Holder
- Malassez/Thomas Hemocytometer
- Blue Trypan
- Micropipettes 20µL and 100µL (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 -3 Cell Passage

1
- Warm the DMEM culture medium, DPBS, and trypsin in a water bath.

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
- 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.

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.

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

Preparation of Cell Suspensions at 2×10^6 Cells/mL

6
- Calculate the volume of DMEM needed to dilute or concentrate the cell suspensions to a concentration of 2×10^6 cells/mL:

  Volume of DMEM to add = Total number of cells / 2×10^6

7 ●
Aspirate the supernatant and add the calculated volume of DMEM to achieve a cell suspension at 2×10^6 cells/mL.
●
Homogenize the cell suspension by pipetting.

Preparation of New T75 or T25 Flasks for KPC A219/iMEF Cell Culture

8 ●
To form 0-40 spheroids: use 1 T25 flask of KPC and 1 T25 flask of iMEF.
●
To form 40-100 spheroids: use 1 T25 flask of KPC and 1 T75 flask of iMEF / 2 T25 flasks of iMEF.

9 ●
For the T75 flask: add 15 mL of DMEM + 300 µL of cell suspension at 2×10^6 cells/mL.
●
For the T25 flask: add 5 mL of DMEM + 100 µL of cell suspension at 2×10^6 cells/mL.

Cell Incubation

10 ●
Observe the plate under the optical microscope.
●
Incubate all flasks in the incubator (37°C, 5% CO2).

Day -1: Addition of TGF Beta and Nanoshuttles

11 **Addition of TGFβ to iMEF Flask 24 Hours Before Co-culture**●
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.

12 **Addition of Nanoshuttles to KPC A219 and iMEF Flasks the Day Before Co-culture**
●
For KPC A219: add 100 µL of Nanoshuttles to the T25 flask.

●
For iMEF: add 50 µL of Nanoshuttles to the T25 flask
and 150 µL of Nanoshuttles to the T75 flask.

Day 0: Co-culture and Spheroid Formation

13 **Cell Passage and**
Preparation of Cell Suspensions at 2×10⁶ Cells/mL Concentration
●
Perform the cell passage and record the concentrations
and cell counts.

14 **Cell Count**●
Prepare the cell suspensions of KPC and iMEF at 2×10⁶
cells/mL concentration and record the volumes of the cell solution.

15 **Preparation of KPC and iMEF Mixing Solution**●
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.

16 ●
Dispense 150 µL of the mixed solution into each well
of the 96-well plate.

17 Incubate
the cells in the 96-magnet plate in the incubator (37°C, 5% CO2) for 6 hours,
then remove the magnet plate.