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FCeM® 3D EXPANSION CULTURE

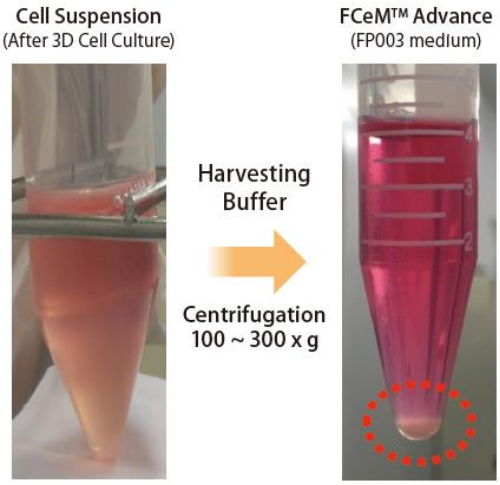
— FCeM® Advance Preparation Kit —

FOR RESEARCH USE ONLY

Nissan Chemical
CORPORATION

1. DESCRIPTION

The FCeM® Advance Preparation Kit consists of all the components needed to transform any 2D medium into a 3D medium for suspension culture of ES and iPS cells. The FP003 polymer, included in the FCeM® Advance Preparation Kit, can be added to any 2D medium to culture ES/iPS cells in suspension. The viscosity of the 3D cell culture medium with FP003 polymer is same as that of water. The FP003 polymer prevents sedimentation, and uniformly disperses and suspends cell spheroids without agitation. The cells can be easily harvested from the medium by our proprietary harvesting buffer followed by centrifugation. Substrate or coating is not required when cells are cultured using 3D medium containing FP003 polymer.



2. COMPONENTS

(1) FP003 Solution † (15 mL × 1 bottle, sterile) †Storage at 2-30 °C, DO NOT freeze.
(2) Conical tube (1 piece, autoclavable, sterile)
(3) Adapter cap (1 piece, autoclavable, sterile)
(4) Plastic flexible needle (1 piece, sterile)
(5) 20 mL syringe (1 piece, sterile)
(6) Harvesting Buffer (110 mL x 1 bottle, sterile)
(7) 100 µm cell strainer

3. APPLICABLE CELL TYPES (for example)

Jurkat E6.1	Human leukaemic T cell lymphoblast
A549	Human caucasian lung carcinoma
NHDF	Human dermal fibroblast
hMSC	Human mesenchymal stem cells
409B2	Human induced pluripotent stem cells
253G1	Human induced pluripotent stem cells
TkDN4-M	Human induced pluripotent stem cells
cPGCs	Chicken primordial germ cells
SMCs	Vascular smooth muscle cells

4. PROCEDURE

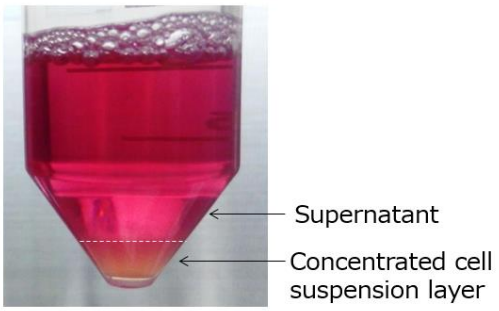
SUSPENSION CELLS

SEEDING (DAY 0)

- The procedure to prepare 3D medium using FP003 Solution can be found in the "Instructions for Use" document included in the FCeM® Advance Preparation Kit.
- Warm the 3D culture medium to 37 ℃.
- Remove the existing medium from the cell suspension after centrifugation.
  (e.g., 200～400×g、3～5 min)
- Add the 3D medium to cell pellet and gently pipette to disperse cell.
  (Seeding density: 5.0×10^4 cells/mL, 10 - 30 mL/vessel, depending on the cell line)
- Transfer the cell suspension to a new culture vessel.
- Culture in a humidified incubator at 37°C, 5% CO_2.

PASSAGING (DAY 3 - 4)

- Transfer the cell suspension to a conical tube.
- **Add 10 vol% of Harvesting Buffer to cell suspension and mix well by 10 times pipetting.**
- **Filtrate the cell suspension to 40 μm cell strainer.**
- Centrifuge at 300 xg, 5 min and set to **SLOW DECEL mode.**
- Remove supernatant from the conical tube so as not to touch concentrated cell suspension layer which is not forming pellet.
- Resuspend cells by 10 mL of fresh 3D medium.
- Measure total number of cells and seed cells onto a new culture vessel at desired cell density.



ADHERENT CELLS

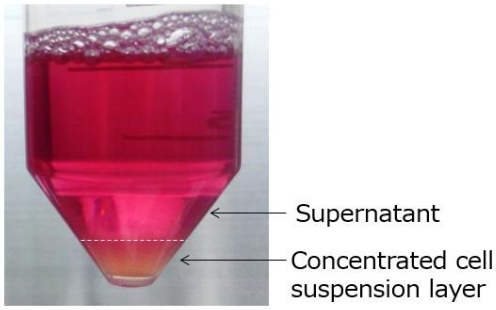
Adherent cell culture using FCeM® Advance 3D culture medium is required low attachment coating cell culture vessel. (e.g., Corning® Ultra-low attachment series: #3261, #3262, #3471, #3473, #3474, #3815, #3814)

SEEDING (DAY 0)

- The procedure to prepare 3D medium using FP003 Solution can be found in the "Instructions for Use" document included in the FCeM® Advance Preparation Kit.
- Warm the 3D culture medium to 37 ℃.
- Remove the existing medium from the cells and wash the cells with PBS (-).
- Add detaching agent (e.g., trypsin) and incubate at 37°C until cells are fully detached from the dish (2–20 min depending on the cell line).
- Resuspend cells in fresh medium, pipette thoroughly to obtain single cell or cell clamp of desired size.
- Remove supernatant after centrifugation (e.g., 200～400×g, 3～5 min).
- Add the 3D medium to cell pellet and gently pipette to disperse cells or clamps. (Seeding density: 5.0×10^4 cells/mL, 10 - 30 mL/vessel, depending on the cell line)
- Transfer the cell suspension to a culture new vessel.
- Culture in a humidified incubator at 37°C, 5% CO_2.

PASSAGING (DAY 3 - 4)

- Transfer the cell suspension to a conical tube.
- **Add 10 vol% of Harvesting Buffer to cell suspension and mix well by 10 times pipetting.**
- Centrifuge at 300 xg, 5 min and set to **SLOW DECEL mode.**



- Remove supernatant from the conical tube so as not to touch concentrated cell suspension layer which is not forming pellet.
- Resuspend cells by 10 mL of fresh 3D medium.

- Measure total number of cells and seed cells onto a new culture vessel at desired cell density.

If you have any questions related to these instructions, encounter problems, or need help, please contact us by email, phone, or fax.

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