Application Note

CELLFLOAT

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Data courtesy of Prof.Uemura, The University of Osaka, Japan

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CellPet 3D-iPS Protocol

Feeder free culture of human iPS cells

MATERIALS

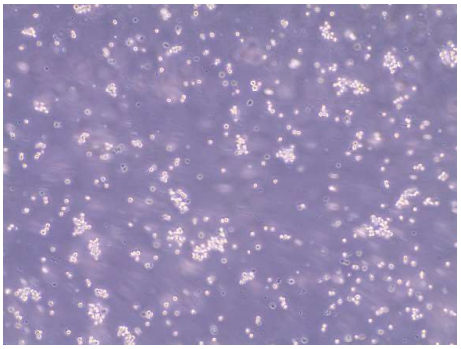
Cell line: 201B7, 253G1, 409B2 (Riken BDR)
ECM coated: iMatrix511 (Matrixome), Matrigel (#356230; Corning)
Medium: mTeSR1 (Stem Cell Technologies), AK02N (Ajinomoto)
StemFlex Medium (ThermoFisher Scientific)

METHODS

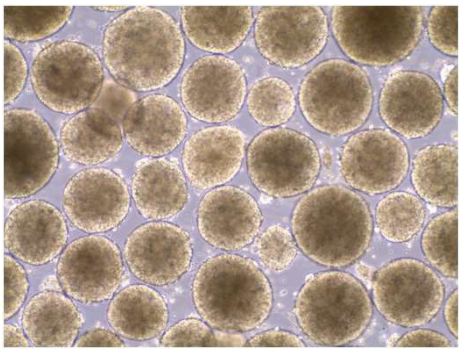
Ready to semi-confluent human iPS cells.
Two times each wash with PBS and aspirate.
Add EDTA solution (5 mM) at room temperature; ex. 60 mm dish: 2 mL
Incubate the dish or plate at 37 ° C for at least 5 min.
Observe the dish or plate, to check loosen cell-cell adhesion.
Removed EDTA solution.
Add culture medium; ex. 60 mm dish: 2 mL
Detach with cell scraper and collect cell cluster with disposable pipette gentry; recommend using 5 mL pipette.
Transfer to microtube at least 300 µL for cell count, clusters dissolved single cells with pipetting. (not seed)
After removing inner plunger, set the female Luer lock cap of outer syringe.
Add culture medium supplemented with Y-27632 (final 10 µM) in syringe vessel.
Seed the cell cluster; recommended using 2 mL pipette.
Attach inner plunger lightly.
Invert the syringe, removed Luer lock cap. And inner air is released by a Luer lock.
[Please attach a needle at Luer lock port for prevention of spray of culture medium]
Set Luer lock cap and removed screw of inner plunger.
The syringe put on CellPet 3D-iPS in 37 °C incubator.
*There are optimum conditions (number of cells, number of rotations) for each cell type.

RESULTS

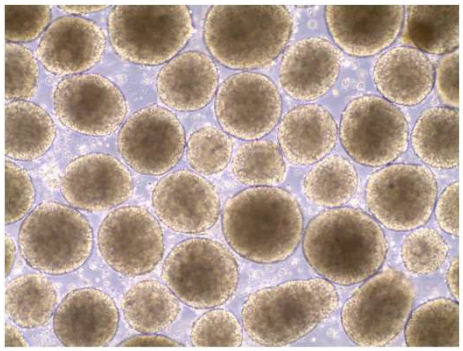
Cell: 253G1, Medium: AK02N,
Rotate Speed: 40 rpm



iPS cells immediately after detachment



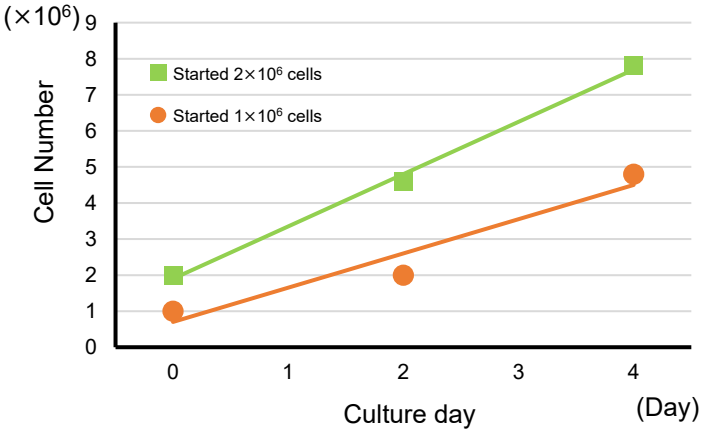
Collected iPS cells
(Seeding cell number: 1×10^5 cells/mL)



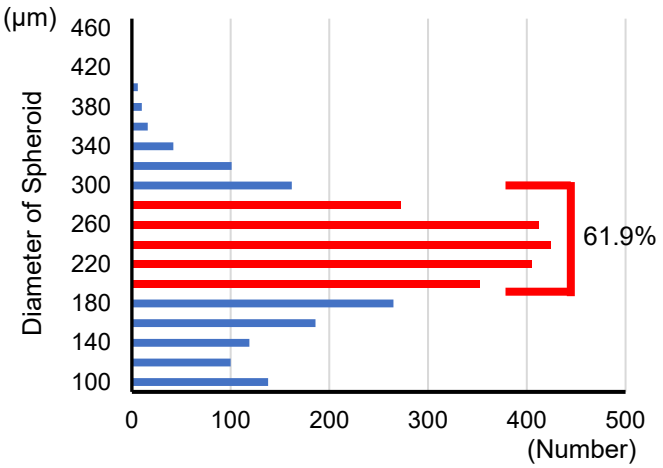
Collected iPS cells
(Seeding cell number: 2×10^5 cells/mL)

Application Note

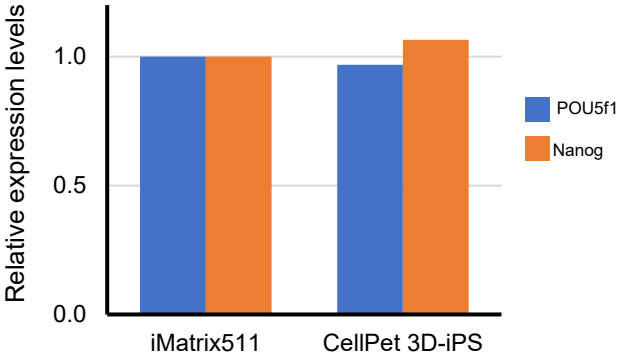
Growth curve



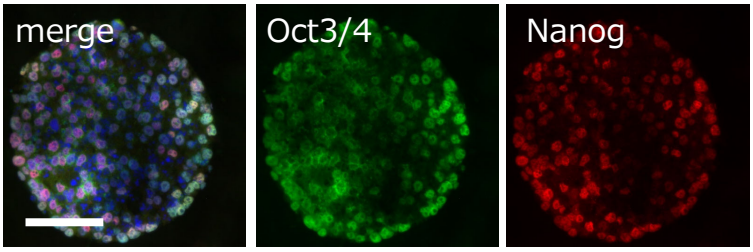
Histogram



RT-qPCR



Immunofluorescence



Bar=100 μm

Karyotypes (Passage 5 and 10) were normal

The CELLFLOAT system allowed us to obtain spheroids that maintained their undifferentiated nature. The size distribution of the spheroids was 61.9% (1865/3013 spheroids) within 200-300 μm in diameter with a mode frequency of 220-240 μm.

DISCUSSION

It was suggested that maintenance of undifferentiated nature by the CELLFLOAT system is possible. The resulting spheroids are uniform.

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