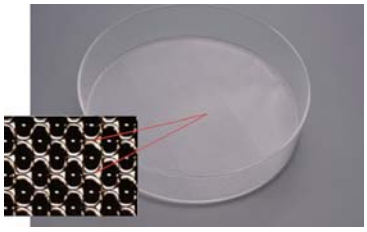
Cell Culture Labware

EZSPHERE® for Spheroid Formation

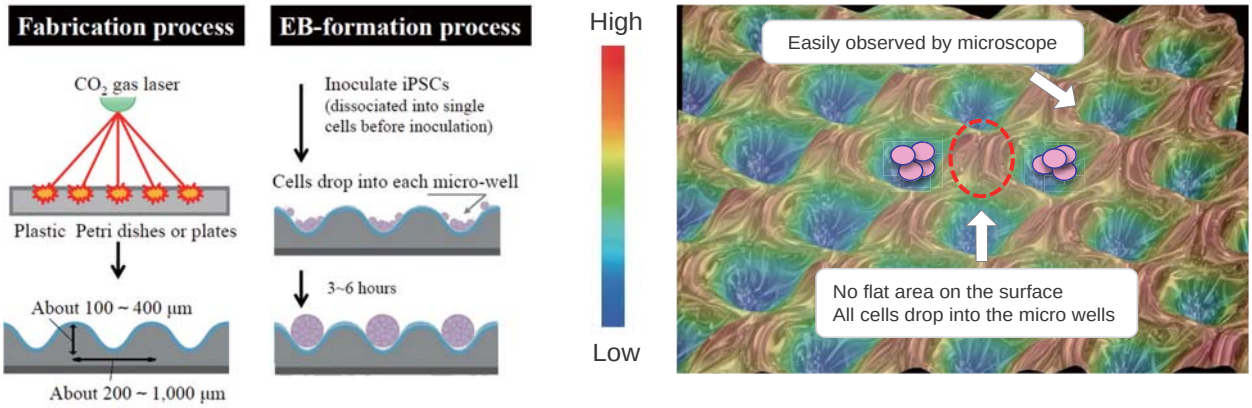
Introduction

Three dimensional (3D) cell culture systems have gained in popularity as invaluable tools in broad applications of cell biology. 3D multi-cellular cell aggregates (spheroids) can be formed by using a low attachment culture surface. However, variability in forming spheroids has been a persistent problem. EZSPHERE is specifically designed to form a large number of uniformly sized spheroids and Embryoid Bodies (EBs).



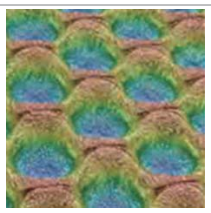
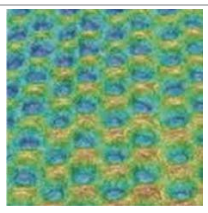
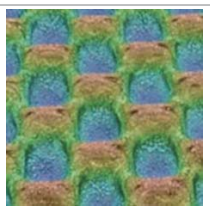
Features

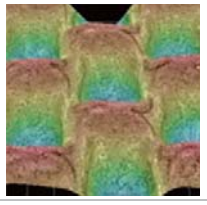
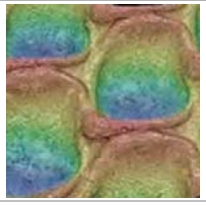
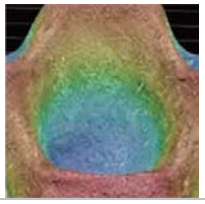
- The surface of EZSPHERE is coated with very low binding 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer.
- EZSPHERE has a lot of evenly designed micro wells on the surface. Inoculated cells drop into the micro wells and form uniformly sized spheroids.
- Spheroids can be formed efficiently in the round shape wells.



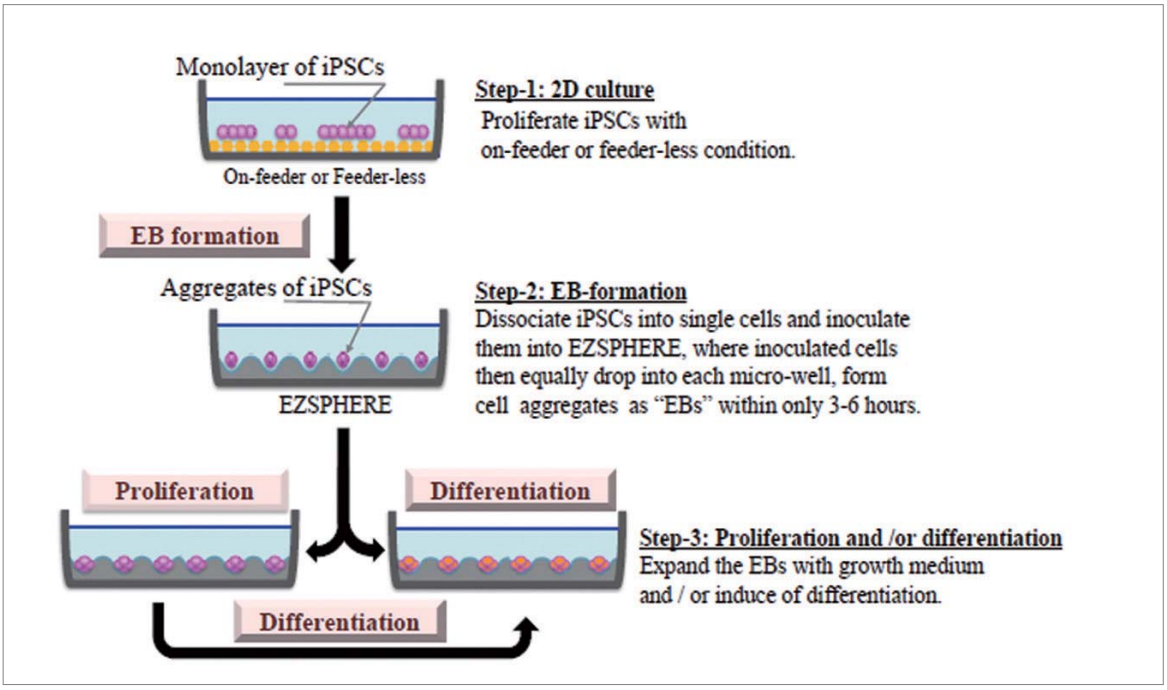
EZ SPHERE is a unique micro-fabricated plastic vessel and very useful for mass-production of EBs with uniform size.

EZSPHERE Variety Pack (35 mm Dish)

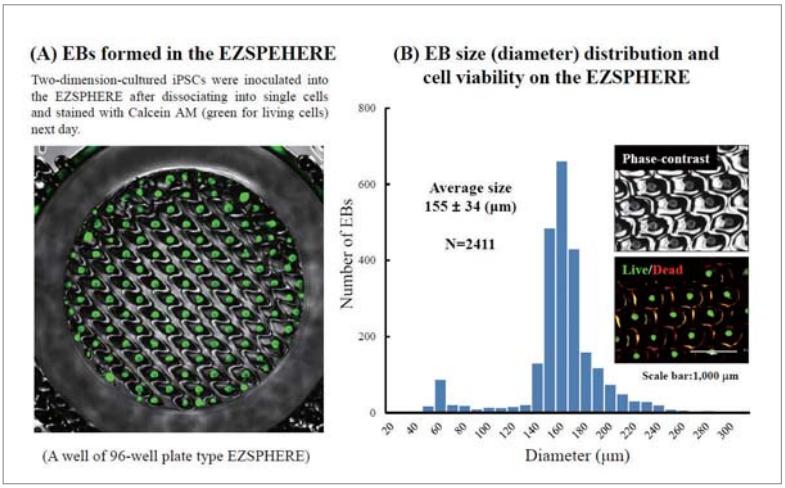
High / Low			
			
Product No.	4000-900SP	4000-901SP	4000-902SP
Diameter (µm)	approx. 500	approx. 200	approx. 500
Depth (µm)	approx. 100	approx. 100	approx. 200
No. of wells	approx. 2,300	approx. 9,200	approx. 2,300

High / Low			
			
Product No.	4000-903SP	4000-904SP	4000-905SP
Diameter (µm)	approx. 800	approx. 800	approx. 1,400
Depth (µm)	approx. 400	approx. 300	approx. 600
No. of wells	approx. 1,000	approx. 600	approx. 200

EB formation, proliferation and differentiation protocols

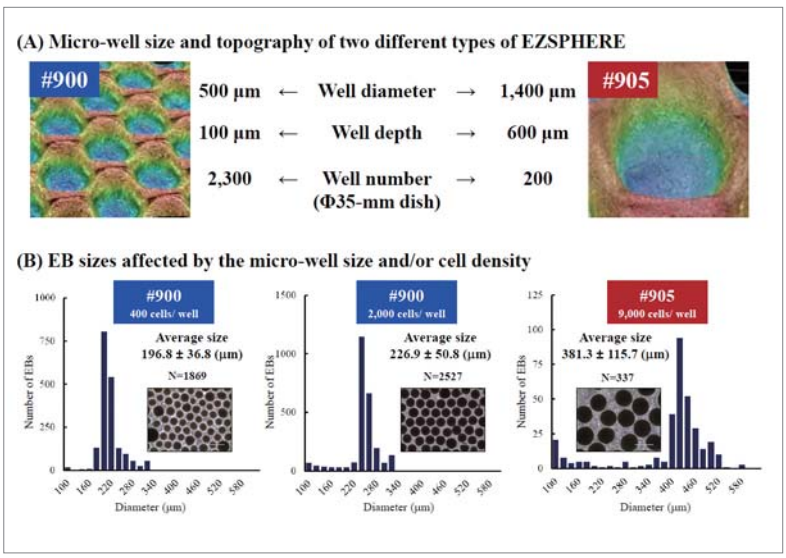


High efficient generation of EBs with uniform size



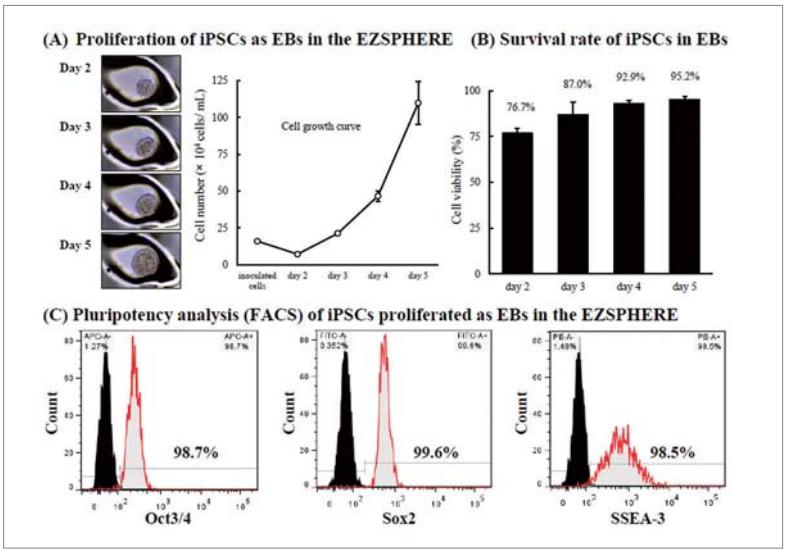
Fluorescence microscopy image of EBs obtained on the EZSPHERE (A). EBs created on the 35 mm dish type EZSPHERE were imaged and analyzed with the digital image analyzing software "Image J" to determine size distribution. Histogram of EB size (diameter) distribution. Fluorescence microscopy revealed that the almost EBs were alive with uniform size (B).

EB-size control with micro-well sizes or inoculating cell demsities



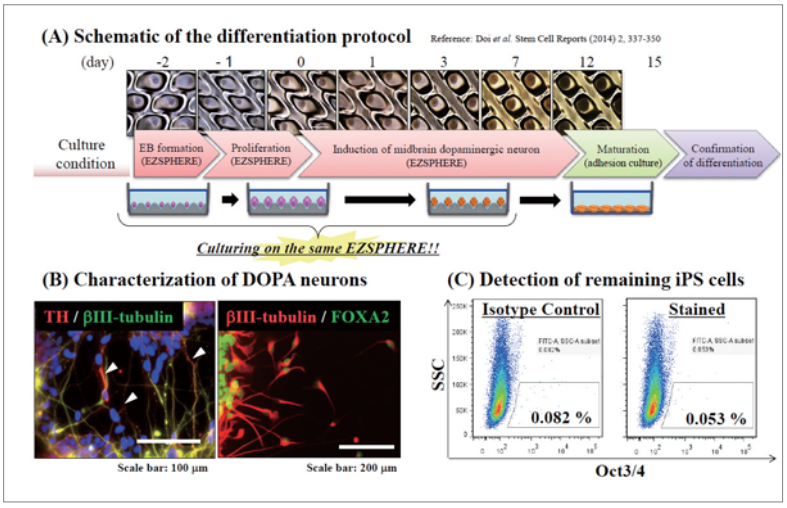
Two types of EZSPHERE #900 and #905 with micro-wells about 500 μm and 1,400 μm in diameter, respectively, were used to analysis the effect of the micro-well size or inoculated cell density on the EB sizes (A). Inoculation of iPSCs as 400 or 2,000 cells per micro-well on the same type of EZSPHERE #900 resulted in the formation of EBs with different sizes, while inoculating iPSCs as 9,000 cells per micro-well on the another EZSPHERE #905 resulted larger size of EBs (B). (Scale bars: 400 μm)

Proliferation of iPSCs with maintaining pluripotency



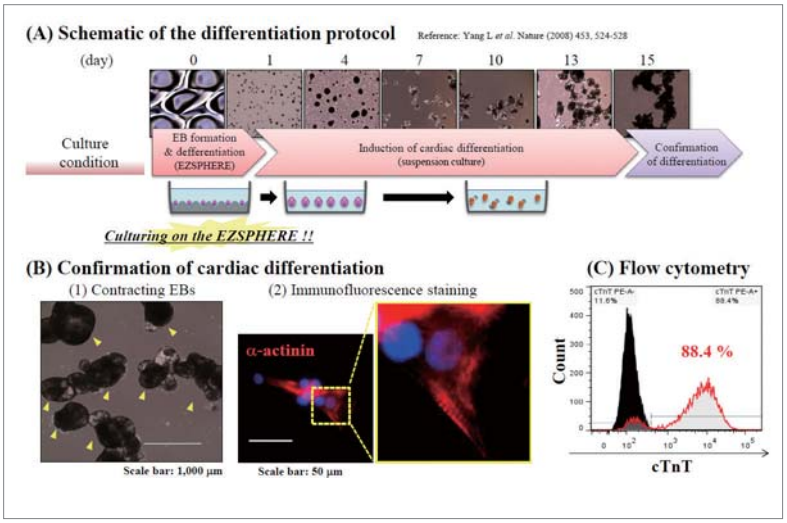
When iPSCs were cultured on EZSPHERE with feeder-free cell culture medium (mTeSR1), the formed EBs could proliferate at a good rate (A) with high viability (B). In the flow cytometry, these cells maintained high capacity of undifferentiated state (C).

Induction of dopaminergic neurons from the EBs



Differentiation of EBs into dopaminergic neuron was attempted by using the EZSPHERE continuously throughout a series of steps from the EB-formation to induction of midbrain dopaminergic neuron (A). Immunofluorescence staining revealed differentiation of the EBs into midbrain neuron, which tyrosin hydroxylase (TH: white arrows) and FOXA2 positive (B). Flow cytometry analysis (FACS) with Oct3/4 antibody indicated that there was almost no iPSCs remained without differentiation (C).

Induction of cardiomyocyte from the EBs



Differentiation of EBs, which prepared on the EZSPHERE, into cardiomyocytes was attempted (A). It was observed that most of EBs showed contracting (indicated with yellow arrow) at day 15 (B)-(1). EBs were dissociated and plated on gelatin coated slide, followed by α-actinin staining and sarcomere alignment (high magnification) was observed (B-2). Flow cytometry for cardiac troponin T (cTnT) positive cells revealed high differentiation efficiency (C).

Reference

1. Zhang RR, Takebe T, Miyazaki L, Takayama M, Koike H, Kimura M, Enomura M, Zheng YW, Sekine K, Taniguchi H Efficient Hepatic Differentiation of Human Induced Pluripotent Stem Cells in a Three-Dimensional Microscale Culture Stem Cells and Tissue Repair Methods in Molecular Biology Volume 1210, 2014, pp 131-141
2. Shimada H, Hashimoto Y, Nakada A, Shigeno K, Nakamura T, Accelerated generation of human induced pluripotent stem cells with retroviral transduction and chemical inhibitors under physiological hypoxia Biochemical and Biophysical Research Communications, Volume 417, Issue 2, Pages 659-664
3. Aikawa N, Suzuki Y, Takaba K A Simple Protocol for the Myocardial Differentiation of Human iPS Cells Biological and Pharmaceutical Bulletin Vol. 38 (2015) No. 7 Pages 1070-1075
4. Matsuura K, Seta H, Haraguchi Y, Alsayegh K, Sekine H, Shimizu T, Hagiwara N, Yamazaki K, Okano T TRPV-1-mediated elimination of residual iPS cells in bioengineered cardiac cell sheet tissues Scientific Reports 6, Article number: 21747 (2016) doi:10.1038/srep21747

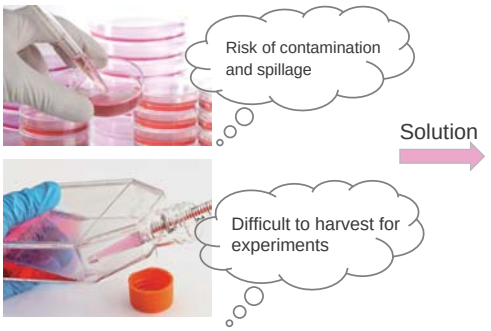
Ordering Information

Product Name	Well Size (µm)	No. of Wells	Product No.	Qty.
EZSPHERE Dish 35 mm	Diameter: 400-500 Depth: 100-200	2,300/dish	4000-900SP	10
EZSPHERE Dish 60 mm		5,300/dish	4010-900SP	10
EZSPHERE Dish 100 mm		14,000/dish	4020-900SP	10
EZSPHERE 6-Well Plate		2,400/well*	4810-900SP	5
EZSPHERE 96-Well Plate		80/well*	4860-900SP	5
EZSPHERE Dish 35 mm Type 902	Diameter: 500, Depth: 200	2,300/dish	4000-902SP	10
EZSPHERE Dish 35 mm Type 903	Diameter: 800, Depth: 400	1,000/dish	4000-903SP	10
EZSPHERE Dish 35 mm Type 904	Diameter: 800 , Depth: 300	600/dish	4000-904SP	10
EZSPHERE Variety Pack (35 mm Dish)	6 PC		4000-9VP	1 set

*Number of spheroid wells per large well

Peel-Off Cell Culture Flask

EZ-OPEN Top FLASK for Easy Cell Retrieval



Sterile polystyrene flasks with filtered cap is commonly used for cell cultures to minimize risk of contamination, however, retrieving cells through the neck of cell culture flask is cumbersome task. EZ-Open Top FLASK with a peel-off cover allows direct access to the culture stocks.

Features

- Peel-off cover allows easy access to the culture surface.
- High quality polystyrene canted-neck flask is tissue culture treated using corona discharge.
- Filtered screw cap contains 2.0 µm hydrophobic membrane.
- Peel-off cover is made of toxin-free PET/PE material.
- Leak-proof with strong heat welding

Ordering Information

Product Name	Surface Area	Capacity	Working Vol.	Product No.	Qty.
EZ-Open Top FLASK 25	25 cm^2	70 ml	5-7.5 ml	3173-025	20
EZ-Open Top FLASK 75	75 cm^2	270 ml	15-22.5 ml	3193-075	20
EZ-Open Top FLASK 150	150 cm^2	600 ml	30-45 ml	3183-150	20

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