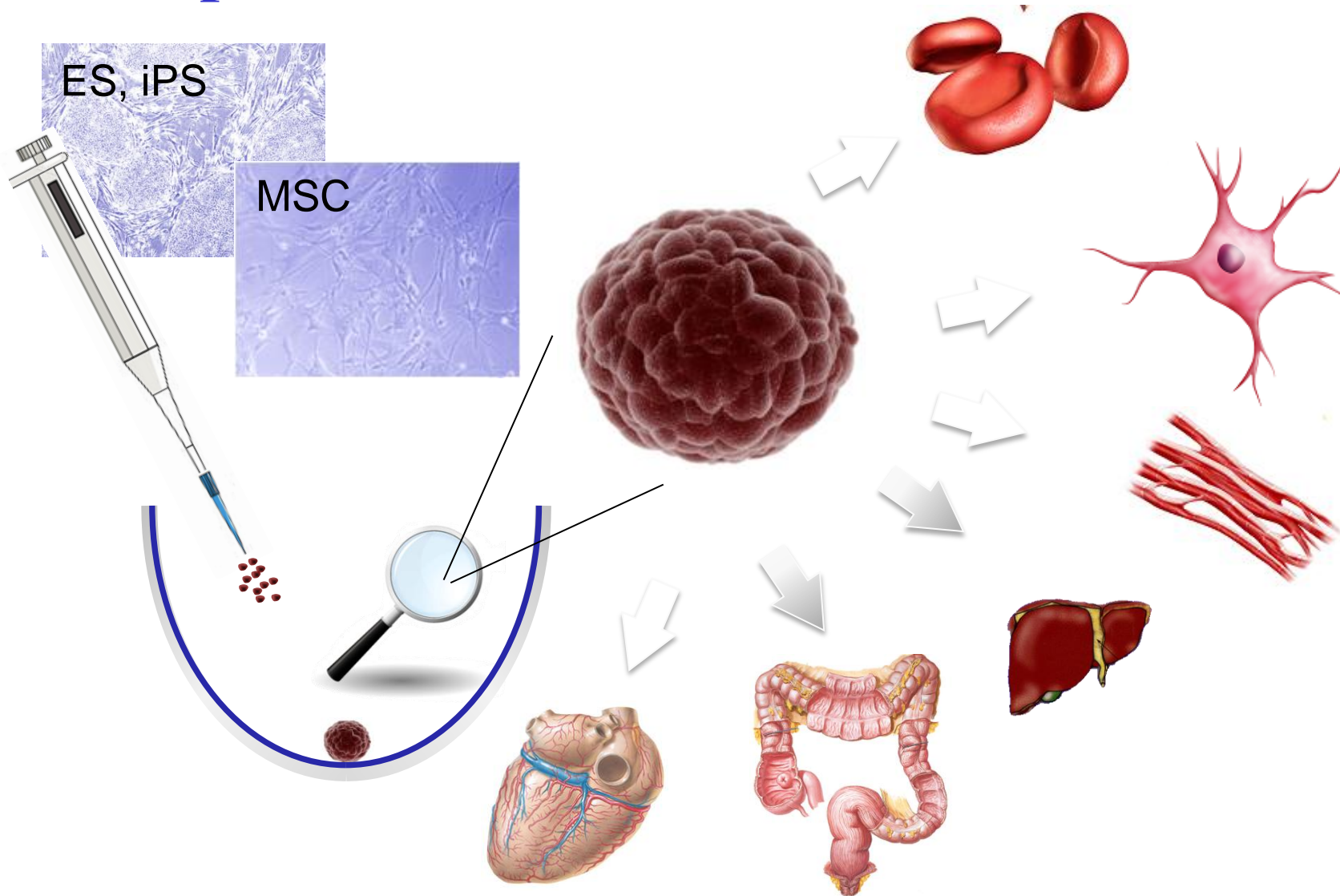




Stem Cell Spheroid Formation with PrimeSurface

Sumitomo Bakelite Co., Ltd.
S-BIO Business Division

Spheroid Formation with PrimeSurface



The Influence of Spheroid Size on Stem Cell Differentiation

The size of spheroids are known to greatly influence stem cell differentiation
(Ref. 1 - 2)



Important to make uniform spheroid

PrimeSurface enables uniform stem cell spheroid formation

[Refernces]

1. Control of human embryonic stem cell colony and aggregate size heterogeneity influences differentiation trajectories. Bauwens, C.L., Peerani, R., Niebruegge, S., Woodhouse, K.A., Kumacheva, E., Husain, M., and Zandstra, P.W. *Stem Cells*, 26, 2300-2310. (2008)
2. Methods for inducing embryoid body formation: in vitro differentiation system of embryonic stem cells. Kurosawa, H., *J Biosci Bioeng*, 103, 389-398. (2007).

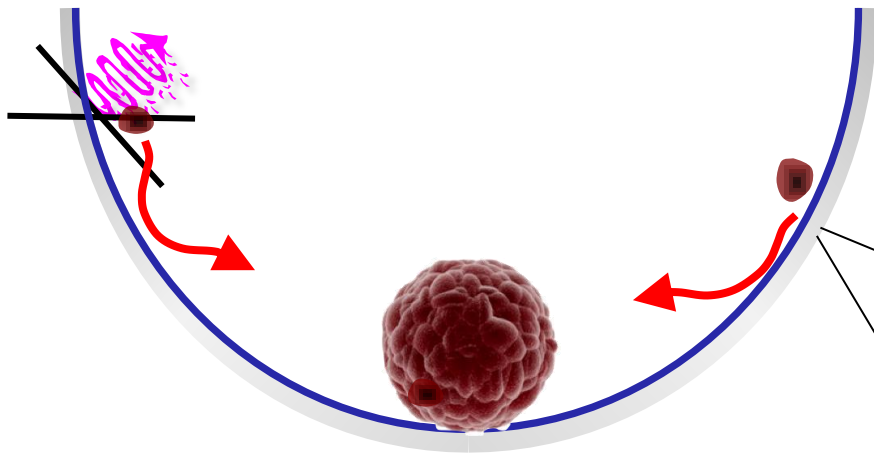
Feature of PrimeSurface

Complete Cell Non-adhesion Surface

Uniform Spheroid Formation

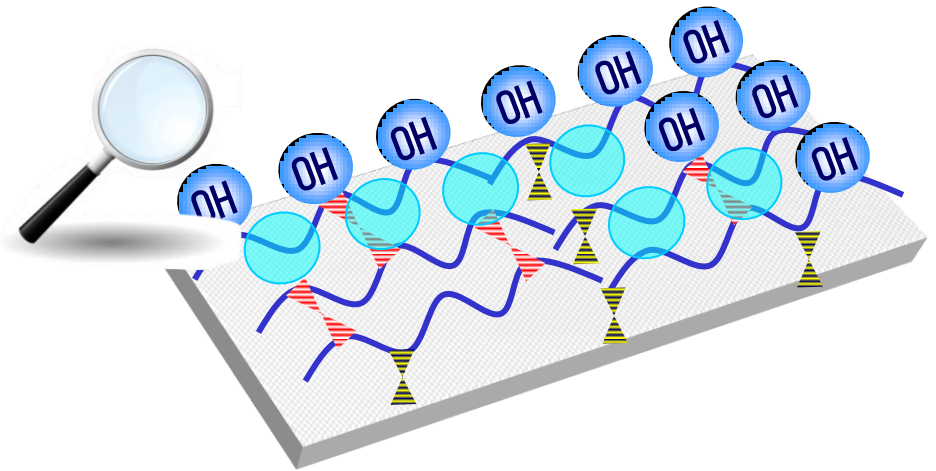
A Variety of Well Shapes

Principle of Spontaneous Spheroid Formation with PrimeSurface

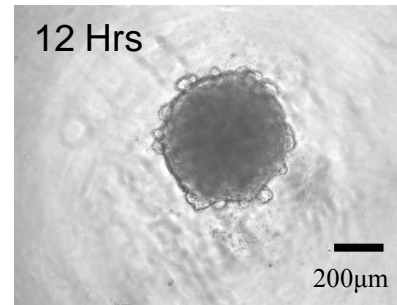
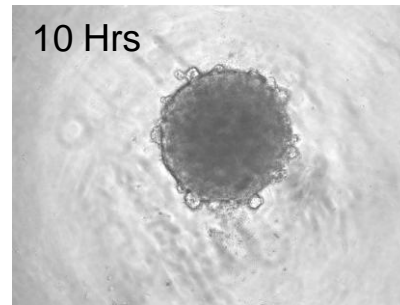
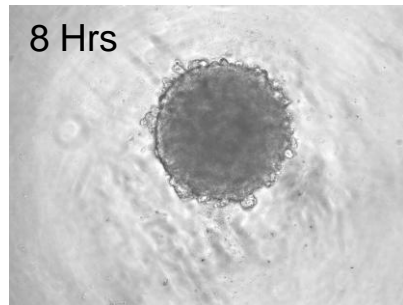
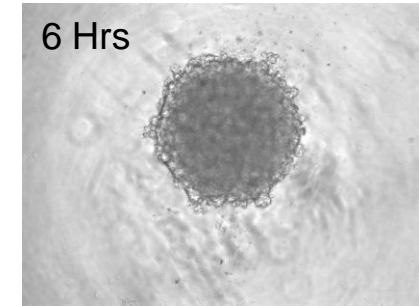
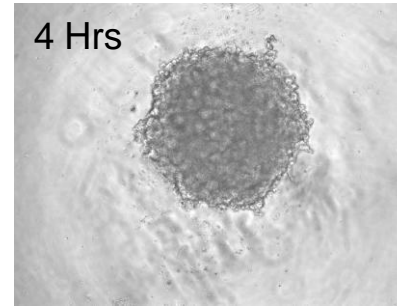
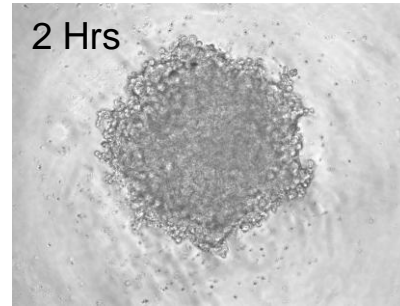
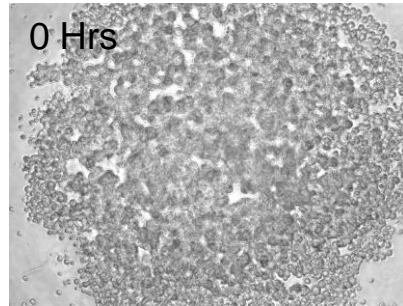


Ultra hydrophilic polymer containing
“high density **-OH** group”

Natural cell spheroid formation
by cell to cell interaction



Time Course Change of Mouse ES Spheroid (EB: Embryoid Body) Formation



【 Microscope 】 : BioStudio
(Correfront Co.,)

【 Culture Conditions 】

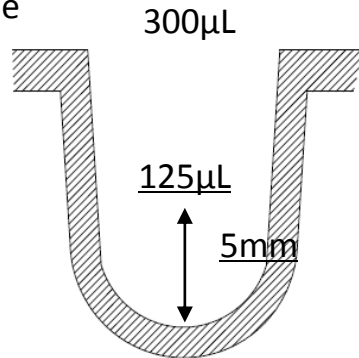
Culture plate : *PrimeSurface MS-9096M*
Kind of cells : Mouse ES Cells 129SV
Seeding density : 1,500 cells/well
Culture medium : DMEM + 4.5 mg/mL Glc.+15% (v/v) heat inactivated FCS + 1%(v/v) NEAA + L-Glutamine(2mM)
+β-Mercaptethanol (110 μM/ml) + 1%(v/v) Pen.-Strep.
Culture environment : 5%CO₂, 37°C

Lineups of PrimeSurface 96 well Plate

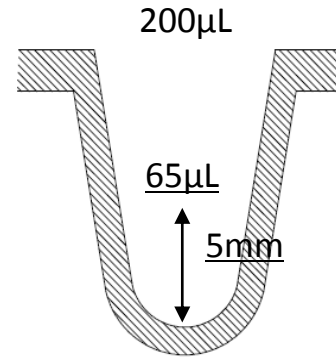
96 well plate

Optimum well shapes according to your cell properties !

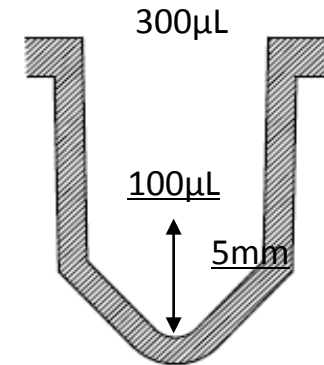
Max Volume



MS-9096U (96 well Clear)
MS-9096W (96 well White)



MS-9096M (96 well Clear)

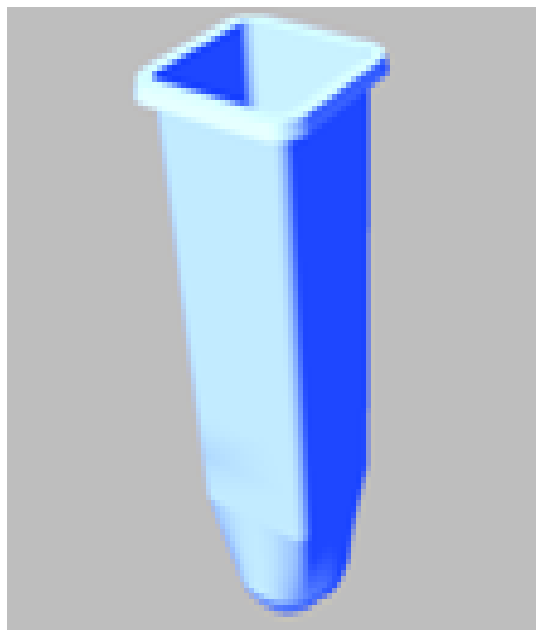


MS-9096V (96 well Clear)

New Lineup ! PrimeSurface 384well Plate







384 well plate

Round shape bottm, Max volume 106 μ L



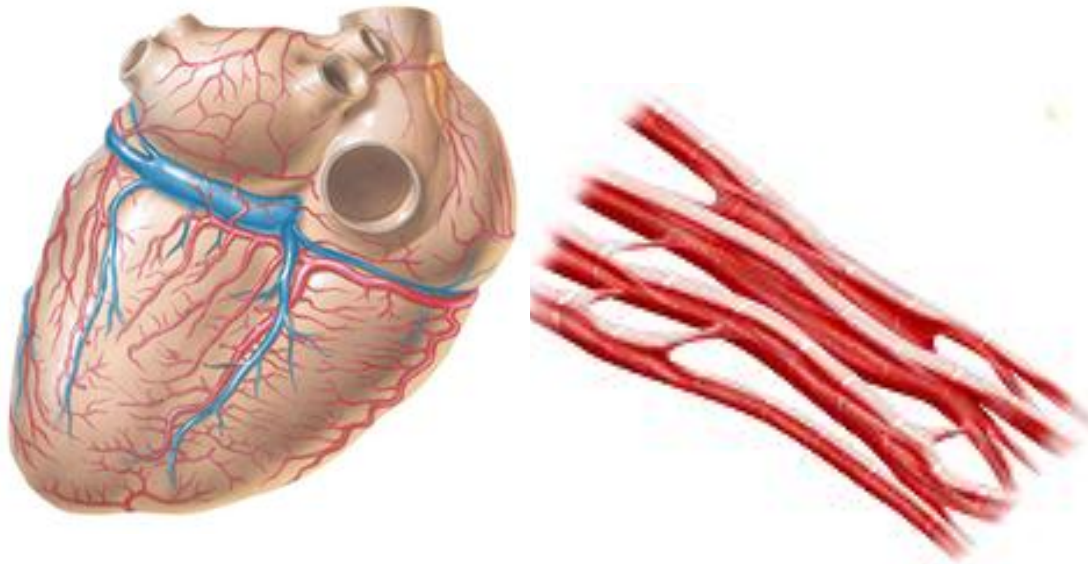
MS-9384U (384 well Clear)
MS-9384W (384 well White)

Lineups of PrimeSurface Multiwell Plate and Dishes

	Cat. No	Product	Wells	Color	Well Bottom	Volume	Package
Multiwell Plate	MS-9024X	PrimeSurface 24 well	24	Clear	Culture area 1.8cm ²	3.4 ml	Individual package 10 plates/cs
	MS-9096U	PrimeSurface 96U	96	Clear		300 μL	Individual package 20 plates/cs
	MS-9096W	PrimeSurface 96W	96	White		300 μL	Individual package 20 plates/cs
	MS-9096M	PrimeSurface 96M	96	Clear		200 μL	Individual package 20 plates/cs
	MS-9096V	PrimeSurface 96V	96	Clear		300 μL	Individual package 20 plates/cs
	MS-9384U	PrimeSurface 384U	384	Clear		106 μL	Individual package 20 plates/cs
	MS-9384W	PrimeSurface 384W	384	White		106 μL	Individual package 20 plates/cs
Dish	MS-9035X	PrimeSurface dish 35mm	—	Clear	Culture area 9cm ²	—	5/package 50/ cs
	MS-9060X	PrimeSurface dish 60mm	—	Clear	Culture area 21cm ²	—	10/package 100/cs
	MS-9090X	PrimeSurface dish 90mm	—	Clear	Culture area 57cm ²	—	10/package 50/cs

Application Examples

Differentiation of mouse ES cells into cardiomyocytes utilizing PrimeSurface MS-9096U (well bottom shape: U)



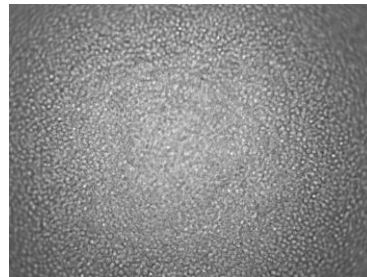
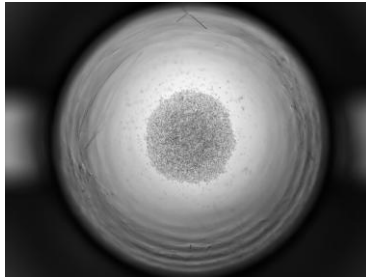
【 References 】

A novel regulator of cardiomyogenesis in pluripotent embryonic cells.

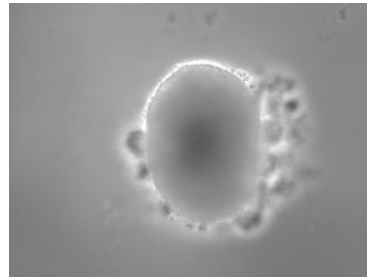
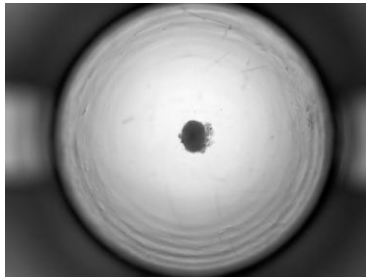
Yasuda S, Hasegawa T, Hosono T, Satoh M, Watanabe K, Ono K, Shimizu S, Hayakawa T, Yamaguchi T, Suzuki K and Sato Y, *Biochem. J.*, 437, 345-355 (2011)

Spheroid formation of human ES cells utilizing PrimeSurface MS-9096M (well bottom shape: \cup)

Day 0



Day 6

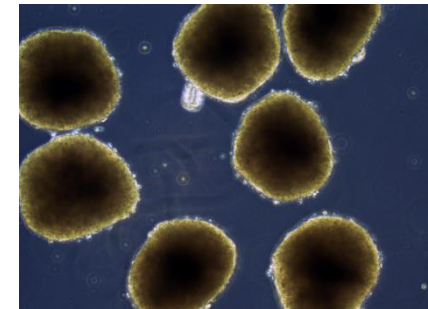


x2

x10

Excellent Size Uniformity

Spheroids were collected from seven wells

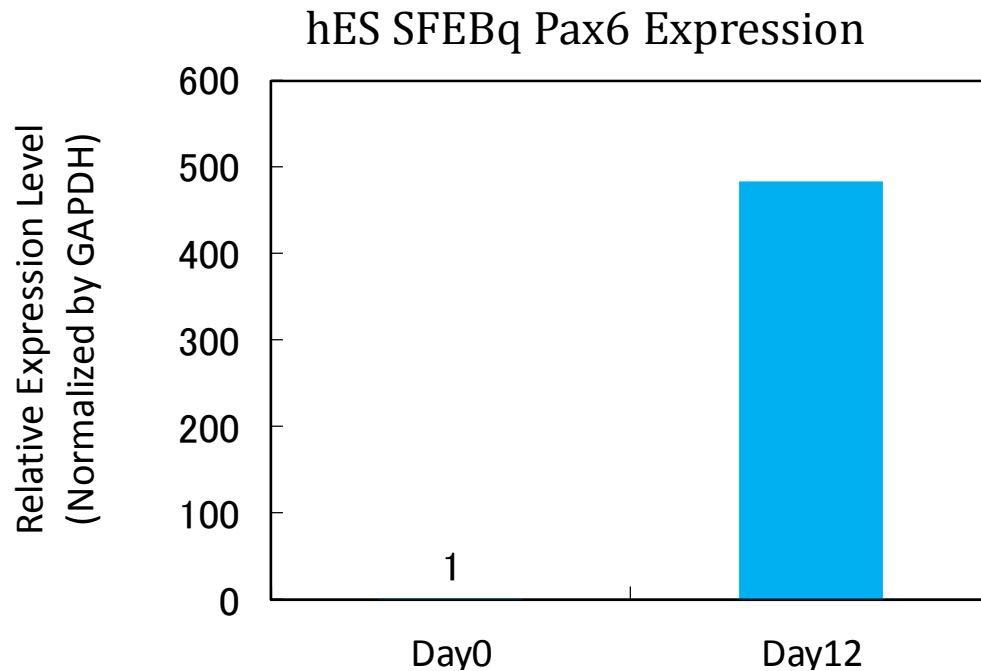


【 Culture Conditions 】

Culture plate : *PrimeSurface MS-9096M*
Kind of cells : *humanES (KhES-1)*
Seeding density : 9,000cells/well
Culture medium : GMEM+KSR+NEAA+2ME + 20uM Y-27632
Culture environment : 5%CO₂, 37°C
Culture period : 6 days

Data provided by Group Director Yoshiki Sasai (M.D., Ph.D.), Division of Human Stem Cell Technology, RIKEN Center for Developmental Biology

Differentiation of human ES cells into Cerebral Cortical Neurons utilizing PrimeSurface MS-9096M (well bottom shape:)



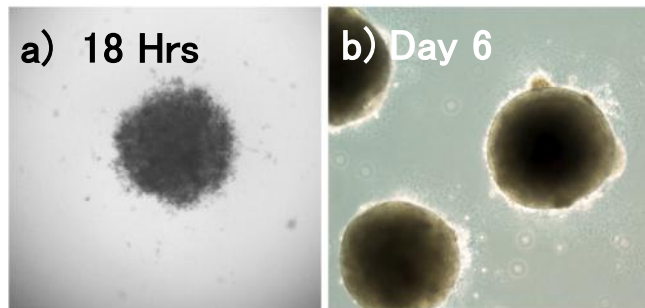
【 Culture Conditions 】

Culture plate : *PrimeSurface MS-9096M*
Kind of cells : *humanES (KhES-1)*
Seeding density : 9,000cells/well
Culture medium : DMEM/F12 + KSR + Y-27632
Culture environment : 5%CO₂, 37°C

Data provided by Group Director Yoshiki Sasai (M.D., Ph.D.), Division of Human Stem Cell Technology, RIKEN Center for Developmental Biology

Marked increase in Pax6 at Day 12 indicates progression of neural differentiation

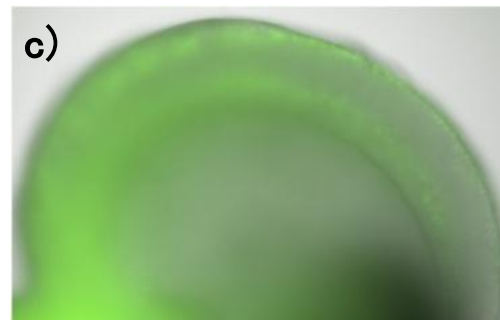
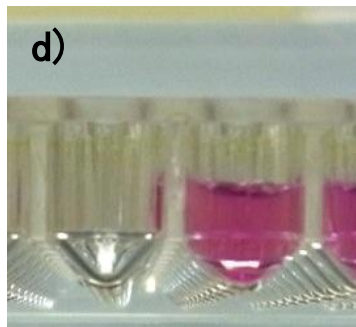
Differentiation of human ES cells into Neural Retina PrimeSurface MS-9096V (well bottom shape:)



Spheroids were collected from three wells

【 Culture Conditions 】

Culture plate : *PrimeSurface MS-9096V*
Kind of cells : *humanES (KhES-1)*
Seeding density : 9,000cells/well
Culture medium : GMEM+KSR+NEAA+2ME+ 20uM Y-27632
Culture environment : 5%CO₂, 37°C



Self-formation of retinal tissue from the aggregate of human ES cells

Images a)-c) provided by Group Director Yoshiki Sasai (M.D., Ph.D.), Division of Human Stem Cell Technology, RIKEN Center for Developmental Biology

【 References 】

Self-Formation of Optic Cups and Storable Stratified Neural Retina from Human ESCs

Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, Saito K, Yonemura S, Eiraku M, Sasai Y
Cell Stem Cell, 10 (6), 771-785 (2012)

References citing PrimeSurface

【MS-9096U】

- A novel regulator of cardiomyogenesis in pluripotent embryonic cells.
Yasuda S, Hasegawa T, Hosono T, Satoh M, Watanabe K, Ono K, Shimizu S, Hayakawa T, Yamaguchi T, Suzuki K and Sato Y, *Biochem. J.*, 437, 345-355 (2011)
- The Transcriptional and Epigenomic Foundations of Ground State Pluripotency
Marks H, Kalkan T, Menafra R, Denissov S, Jones K, Hofemeister H, Nichols J, Kranz A, Stewart F, Smith A, Stunnenberg H. G, *Cell*, 149, 590-604 (2012)

【MS-9096V】

- Self-Formation of Optic Cups and Storable Stratified Neural Retina from Human ESCs
Nakano T, Ando S, Takata N, Kawada M, Muguruma K, Sekiguchi K, Saito K, Yonemura S, Eiraku M, Sasai Y
Cell Stem Cell, 10 (6), 771-785 (2012)

【MS-9096W】

- Evaluation of novel high-throughput embryonic stem cell tests with new molecular markers for screening embryotoxic chemicals in vitro
Suzuki N, Ando S, Yamashita N, Horie N, Saito K, *Toxicol. Sci.*, 124 (2), 460-471 (2011)

【MS-9035X (35mm Dish)】

- Generation of induced pluripotent stem cells from human adipose-derived stem cells without c-MYC.
Aoki T, Ohnishi H, Oda Y, Tadokoro M, Sasao M, Kato H, Hattori K, Ohgushi H. *Tissue Eng. Part A*. 16 (7), 2197-2206 (2010)
- Induction of pluripotent stem cells from human third molar mesenchymal stromal cells.
Oda Y, Yoshimura Y, Ohnishi H, Tadokoro M, Katsube Y, Sasao M, Kubo Y, Hattori K, Saito S, Horimoto K, Yuba S, Ohgushi H, *J. Biol. Chem.*, 285 (38), 29270-29278 (2010)

Appendix

Comparison of PrimeSurface (MS-9096U)
with Other Competitive Products

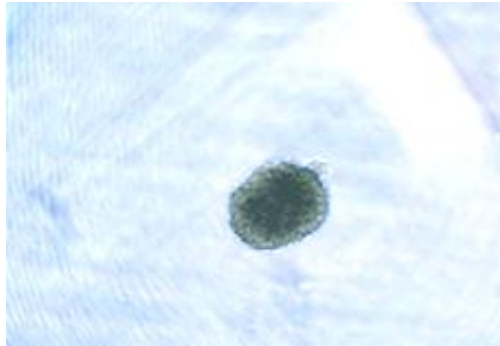
Example of Comparison

Experimental Conditions

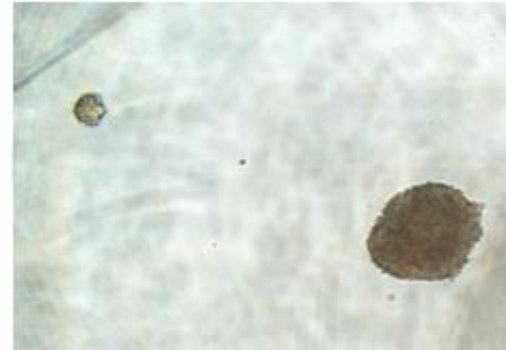
- Used cell : HepG2
- Medium : DMEM Low Glc. +10% FCS
- Number of cells seeded : 1,000 cells/100 μ L/well
- Culture period : 3 days

Evaluation Criteria

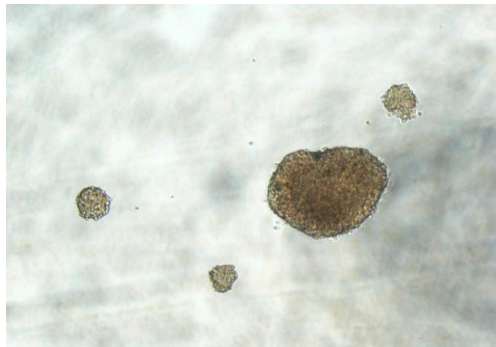
The test results were classified below into four grades according to their size distribution in 96 wells.



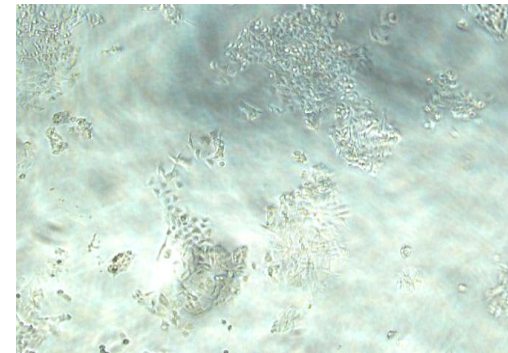
Grade A: Single spheroid in one well



Grade B: Large spheroid with one small spheroid



Grade C: Large spheroids with a few small spheroids



Grade D: Cells were adhered on the inner wall of the well and no cell spheroids were observed

Excellent A > B > C > D Bad

Evaluation Result

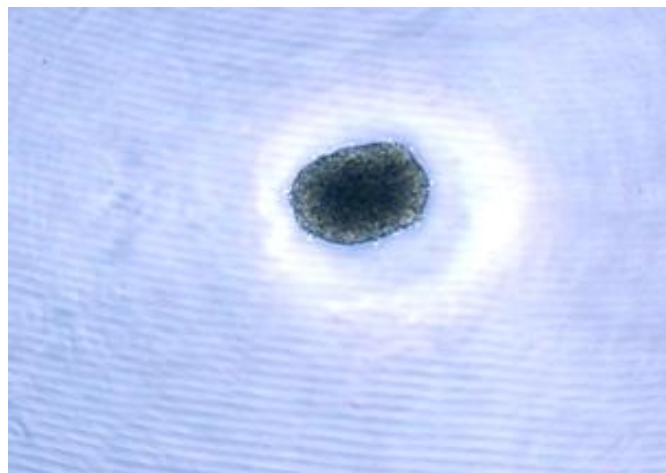
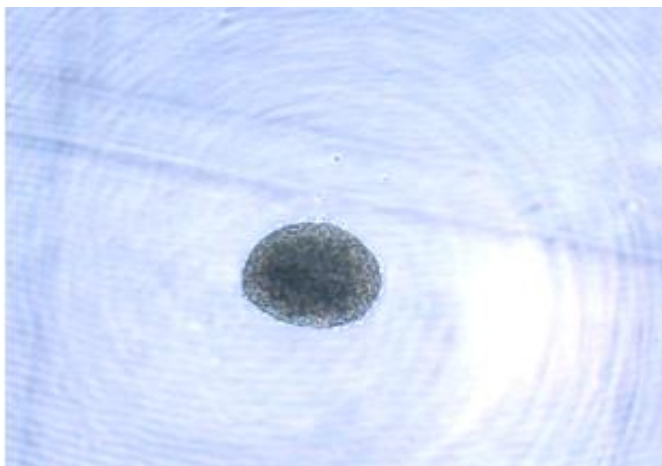
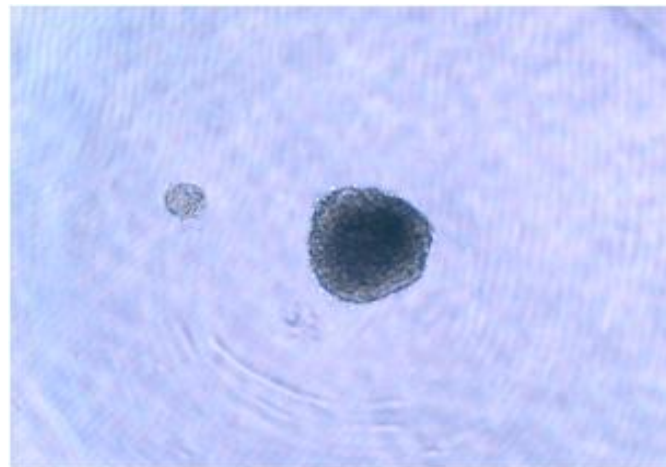
PrimeSurface showed by far the best performance among the four plates

Manufacturer	Products	Grade			
		A	B	C	D
Sumitomo Co. Ltd.	PrimeSurface MS-9096U	95	1	0	0
Company X	Product X	87	6	3	0
Company Y	Product Y	83	9	4	0
Company Z	Product Z	0	0	49	47

1. PrimeSurface96U plate
Sumitomo Bakelite Co. Ltd.

Grade A (Excellent) : 95/96 well

Grade B : 1/96 well



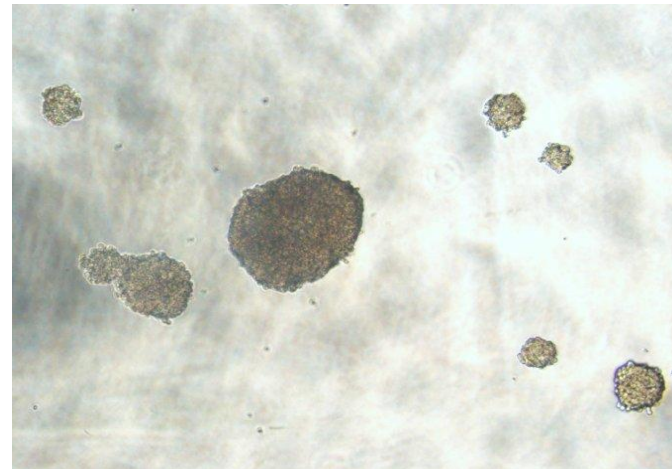
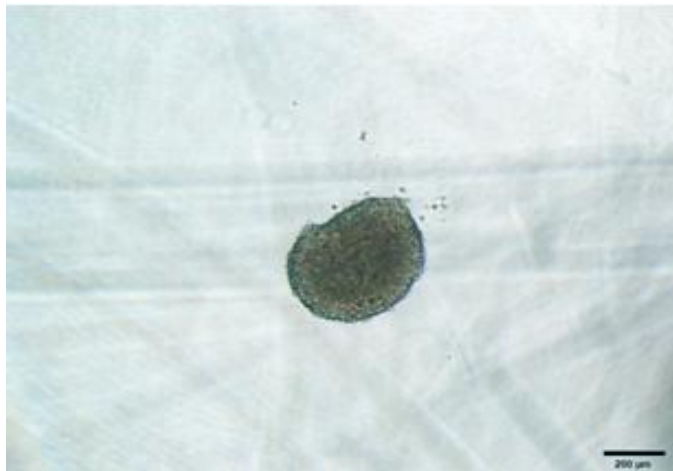
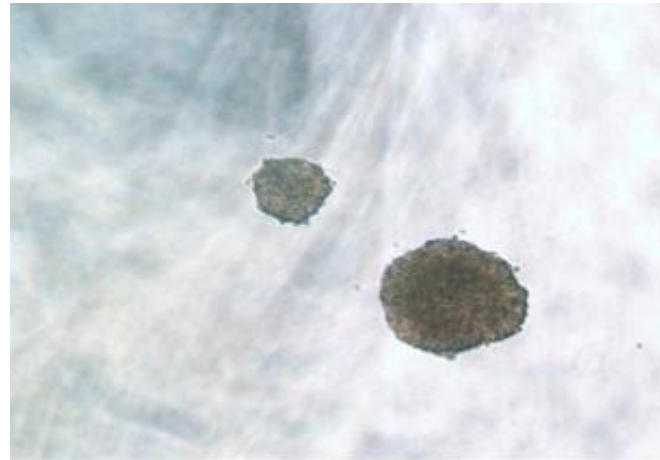
The best performance was observed among the four plates.

2. Product X

Grade A: 87/96 wells

Grade B: 6/96 wells

Grade C: 3/96 wells

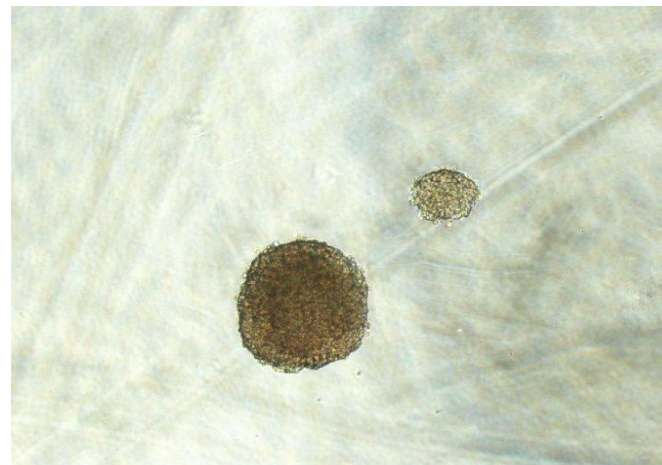


3. Product Y

Grade A: 83/96 wells

Grade B: 9/96 wells

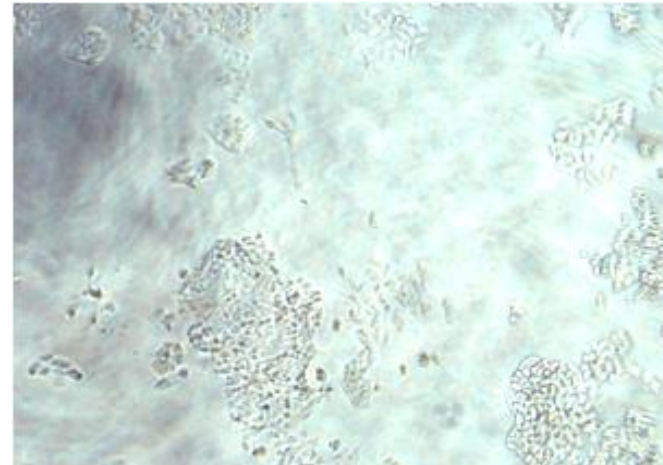
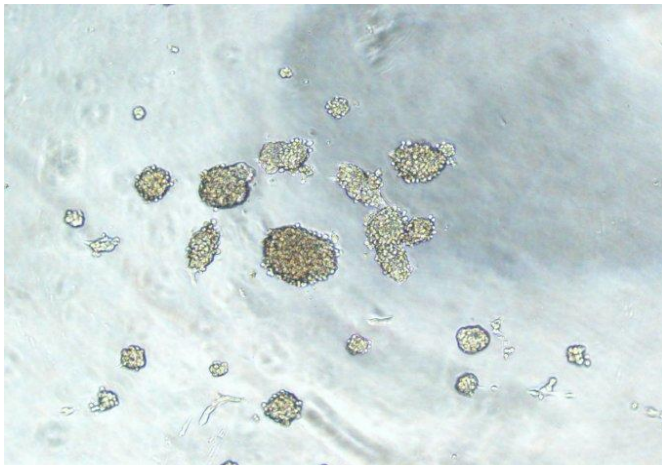
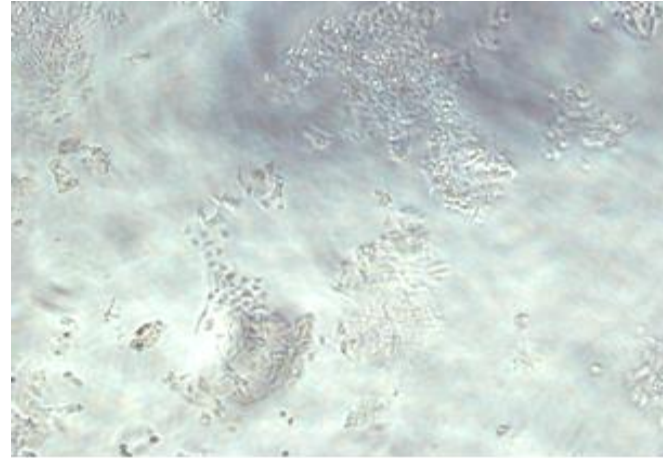
Grade C: 4/96 wells



4. Product Z

Grade C: 49/96 wells

Grade D: 47/96 wells



Cells were adhered in all the wells. This plate is not appropriate for uniform size spheroid formation.

Other Information

- PrimeSurface can be stored at room temperature.
- The shelf life of PrimeSurface is two years after production.

“Sumitomo Bakelite Co. Ltd.”, offers a variety of products based on its advanced plastic and polymer technology for the pharmaceutical researcher engaged in cell based assaying.

We will customize products at your request....

【 Contact Information 】

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<http://www.sumibe.co.jp/english/product/s-bio/index.html>