StemFit™ Basic05

Animal Origin-Free
Clinical-Ready
hPSC Suspension Culture Medium

01

Scalable Expansion

02

Differentiation Potential

03

Seamless 2D to 3D Culture

Ver.2

StemFit™ Basic05

Contents

- Suspension Culture Media - What is StemFit™ Basic05? - 3
- Expansion Capability and Its Consistency Across Cell Lines 4
- Viability and Uniform Spheroid 5
- Flexible Workflow 6
- Scalability and Usability Among Various Culture Reactors 7
- Differentiation Potential 8
- 2D Performance 10
- FAQ 11

Suspension Culture Medium
- What is StemFit™ Basic05? -

In recent years, as cell therapy moves toward practical application, scalable manufacturing has become a key challenge. To enable widespread adoption, significant cost reduction is essential. Currently, adherent culture is commonly used to expand iPSCs, but it is limited by surface area and requires scale-out or scale-up for large-scale production.
StemFit™ Basic05 is designed to address these needs, enabling high growth and uniform spheroid formation in suspension culture. Furthermore, it allows a seamless transition from 2D adherent to 3D suspension culture using a single medium, reducing the need for re-optimization and improving process efficiency and reproducibility.
In addition, StemFit™ is an Animal Origin-Free (AOF) medium designed for clinical applications, enabling consistent use from research through clinical manufacturing. By minimizing changes in materials and process parameters, it helps to reduce variability and facilitates a smoother transition to clinical-scale production.

Key Benefits

① **Scalable Expansion**
Enables scale-up through suspension culture. Supports culture process design from early-stage development with manufacturing in mind.

② **Differentiation Potential**
PSCs cultured in Basic05 retain their differentiation capability, and have been confirmed to be capable of differentiation directly from 3D aggregates.

③ **Seamless 2D to 3D Transition**
Basic05 supports both 2D and 3D culture. The use of a single medium across culture modes minimizes process re-development and optimization associated with media changes.

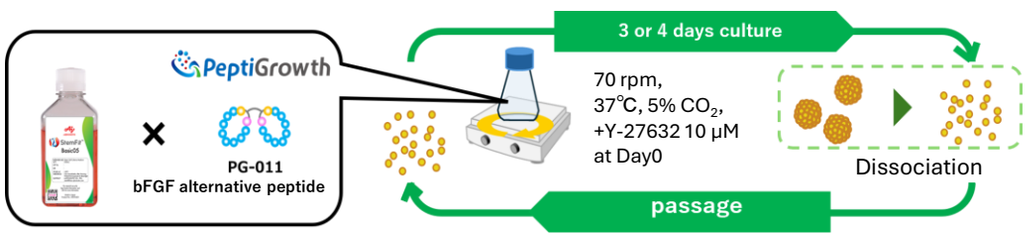
④ **Animal Origin-Free**
An Animal Origin-Free (AOF) formulation reduces risks associated with undefined components. Designed to meet the requirements for **clinical applications**, enabling consistent use from research through clinical development.

Expansion Capability and Its Consistency Across Cell Lines

Basic05 supports strong and reproducible expansion of human iPSCs across multiple cell lines in 3D culture. High growth performance is maintained over passages, while pluripotency remains stable.

(A) Method

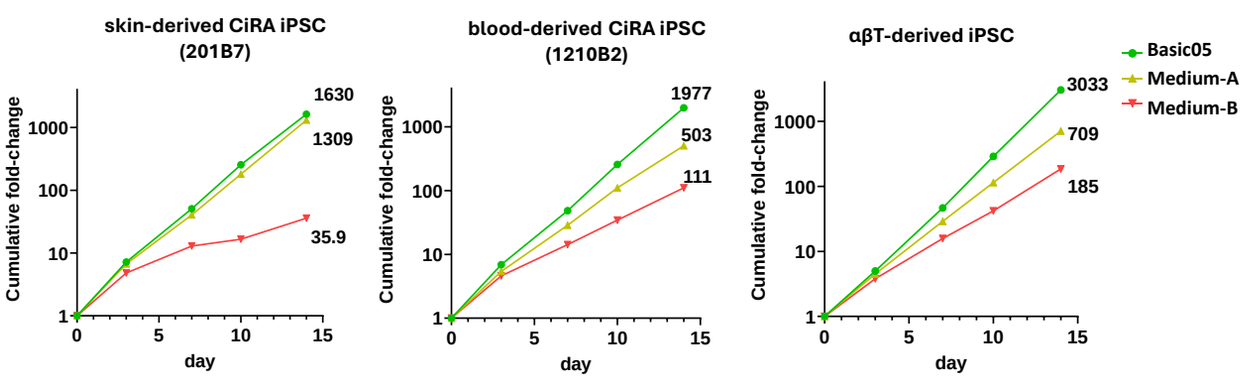
Using Basic05 and two competitor media (Medium-A and Medium-B), three human iPSC lines were cultured in 3D under orbital shaking in 125 mL flasks for up to four passages, and growth rate and pluripotency were evaluated.



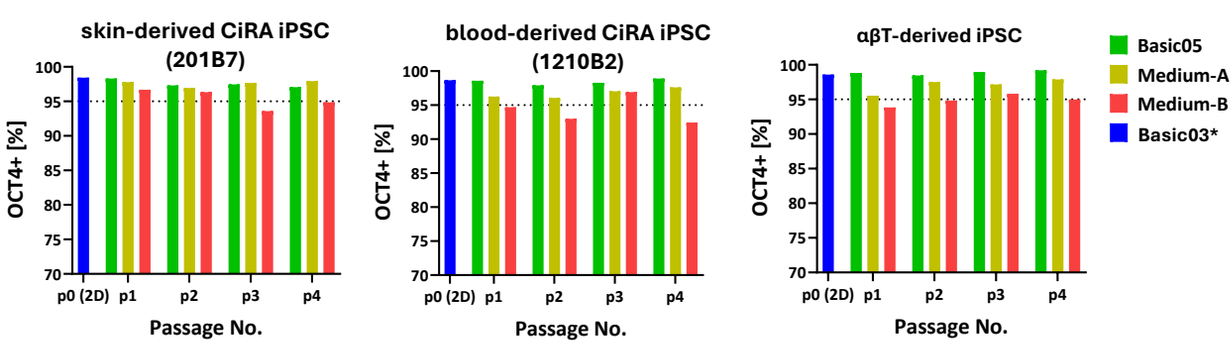
Passage	1				2				3			4			
Days in Culture	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Operations	P	M	M	P	M	-	M	P	M	M	P	M	-	M	P
Seeding density	1.5×10^5 cells/mL														

P: Passage、 **M**: Medium change (50%)、 **-**: Skip

(B) Cumulative fold expansion in 3D culture of each human iPSC line (four passages).



(C) OCT4-positive rate of each human iPSC line (at each passage).

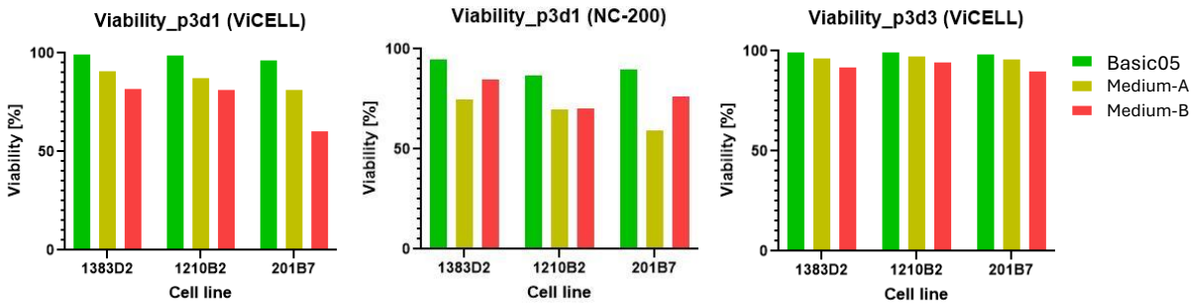


Basic03 refers to StemFit™ Basic03, a 2D culture medium for hPSC expansion.

Viability and Uniform Spheroid

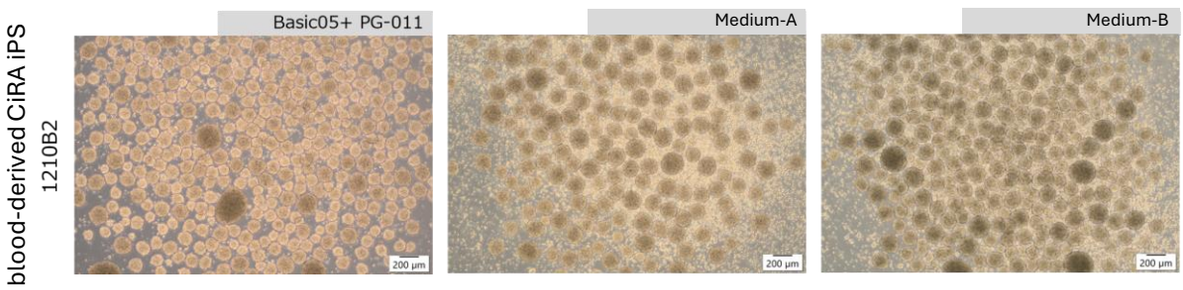
High cell viability and uniform spheroid formation are critical for stable suspension culture and scalable processes.
With Basic05, high cell viability and uniform spheroid formation were observed across multiple human iPSC lines, along with maintenance of a normal karyotype. These results demonstrate that Basic05 enables stable and reproducible culture while maintaining cell quality.

(A) High cell viability across multiple human iPSC lines
Cell viability was evaluated using Vi-CELL and NC-200 across Basic05 and competitor media (Medium-A and Medium-B). Basic05 demonstrated high viability at both Day 1 and Day 3 in cells at passage 3.



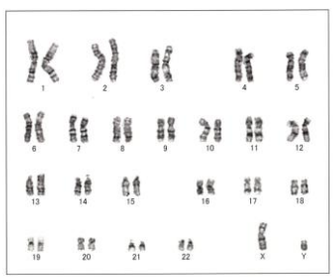
Cell lines:
1383D2 = skin-derived CiRA iPSC, 1210B2 = blood-derived CiRA iPSC, 201B7=skin-derived CiRA iPSC

(B) Uniform spheroid formation
Basic05 enables uniform spheroid formation with fewer dead cells (Passage 3, Day 1).



(C) Normal karyotype analysis of cells cultured in Basic05
Karyotype analysis at Passage 4 confirmed a normal karyotype in all three iPSC lines cultured in Basic05.

Cell Lines	P4
skin-derived CiRA iPSC 1383D2	46, XY [20]
blood-derived CiRA iPS 1210B2	46, XX, inv(9) (p12q13) [20]
αβT-derived iPSC	46, XX [20]

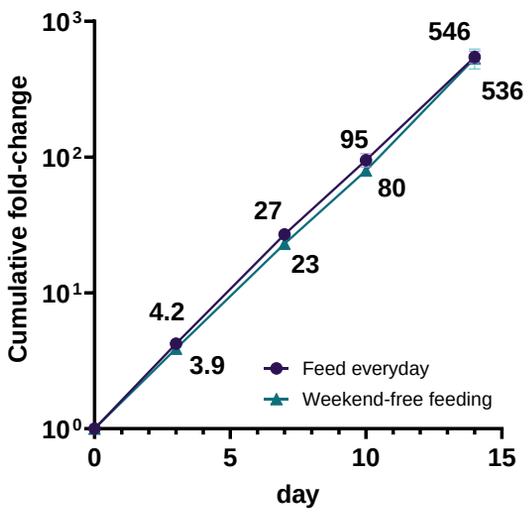


Flexible Workflow

Basic05 provides flexibility in culture scheduling and improves operational efficiency.
Comparable expansion was achieved even when weekend feeding was skipped, in addition to daily feeding conditions. This allows flexible culture schedules based on experimental needs and operational constraints.
As a result, weekend work and labor can be reduced while maintaining stable cell growth. This flexibility is supported not only by the performance of Basic05, but also by the excellent thermal stability of PG-011.

(A) Cumulative cell expansion under different medium change frequencies

Seeding density: 1.5x 10^5 cells/mL (3 day, 4 day)



Feeding Schedules

F: 50% MC F+: 75% MC **P**: Passage

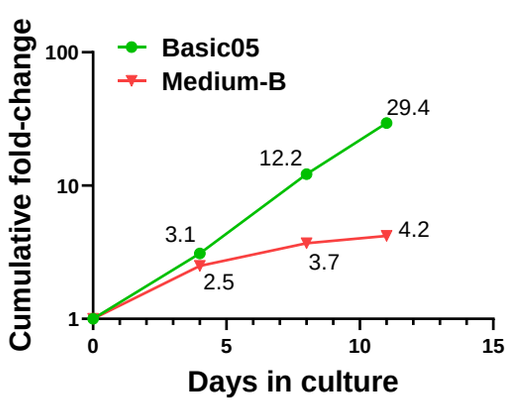
	Sun.	Mon.	Tue.	Wed.	Thu.	Fri.	Sat.
Feed everyday			P	F	F	P	F
	F	F	P	F	F	P	F
	F	F	P				
Weekend-free feeding			P	F	-	P	-
	-	F+	P	F	-	P	-
	-	F+	P				

Robust Cell Expansion in Spinner Flask (30 mL)

Human iPSC line 1383D2 was cultured in suspension using a 30 mL spinner flask.
Basic05 supplemented with PG-011 demonstrated stable cell growth over three passages, reaching a cumulative expansion of approximately 29-fold.

Reactor	Spinner flask 30 mL
	
Passage Number	3 passages
Days in Culture	3 or 4 days
Medium	Basic05
Seeding Density	2.0×10^5 cells/mL
Medium Volume	30 mL

(A) 3-passage cumulative fold expansion (vs. competitor media)



Scalability and Usability Among Various Culture Reactors

Basic05 is compatible with a wide range of bioreactors, enabling flexible, platform-independent process development and a smooth transition from research to manufacturing scale, while minimizing re-optimization and technology transfer burden.
Using Basic05, a seamless 2D-to-3D culture process was established and scaled up to 1.5 L, achieving consistent 3–7 fold expansion per passage and stable cell growth from approximately 6 x10^6 to 1.2 × 10^9 cells (~200-fold expansion), while maintaining uniform aggregates and high pluripotency.

(A) Scalability Test Method

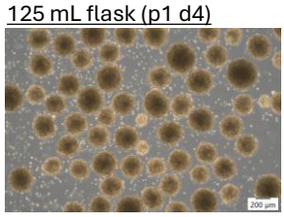
Over four passages (20 days), scale-up was evaluated by starting from 2D culture and stepwise transitioning to a shaker platform, spinner flask, and vertical-wheel reactor.

Passage No.	0 (2D pre-culture)						1				2				3			4			
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Operation	P	M^{++}	M^{+++}	-	-	P	M	-	M	P	F	-	M	P	F	M	P	-	M	M	P
Culture vessel	10 cm dish						125 mL flask				PBS-Mini 0.1 L				PBS-Mini 0.5 L			1.5 L reactor			
Medium	StemFit™ Basic05																				
Seeding density	1.3×10^4 cells/mL						1.5×10^5 cells/mL														
Volume（mL）	9						20				60 -> 100				300 -> 500			1500			

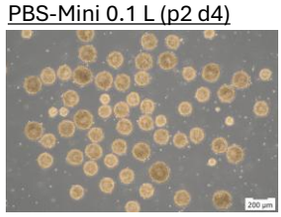
P: Passage, M: Medium change (50%), M^{++}: Medium change (100%), M^{+++}: Medium change (200%) , **F**: Medium feed , -: Skip

(B) Aggregate morphology of human iPSC line (1383D2) at the end of each passage

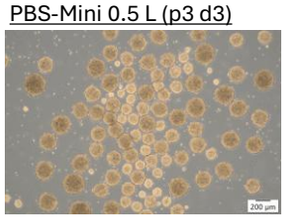
125 mL flask (p1 d4)



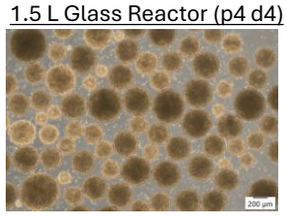
PBS-Mini 0.1 L (p2 d4)



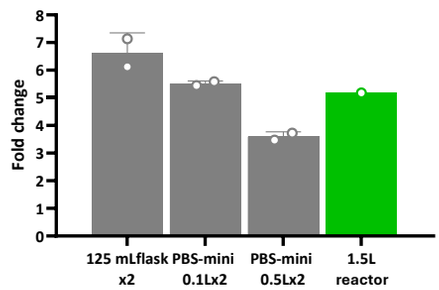
PBS-Mini 0.5 L (p3 d3)



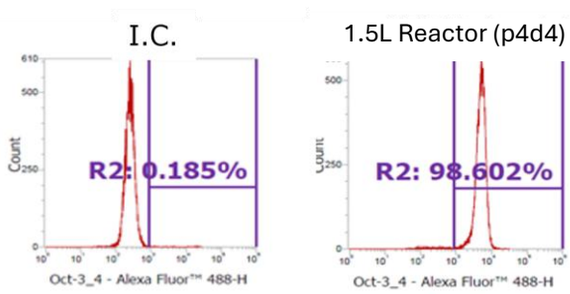
1.5 L Glass Reactor (p4 d4)



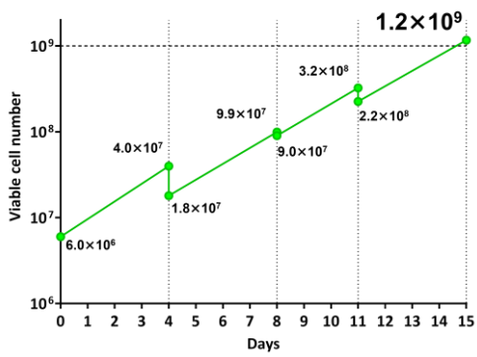
(C) Cell growth rate across suspension culture vessels



(D) Expression analysis of the pluripotency marker OCT4

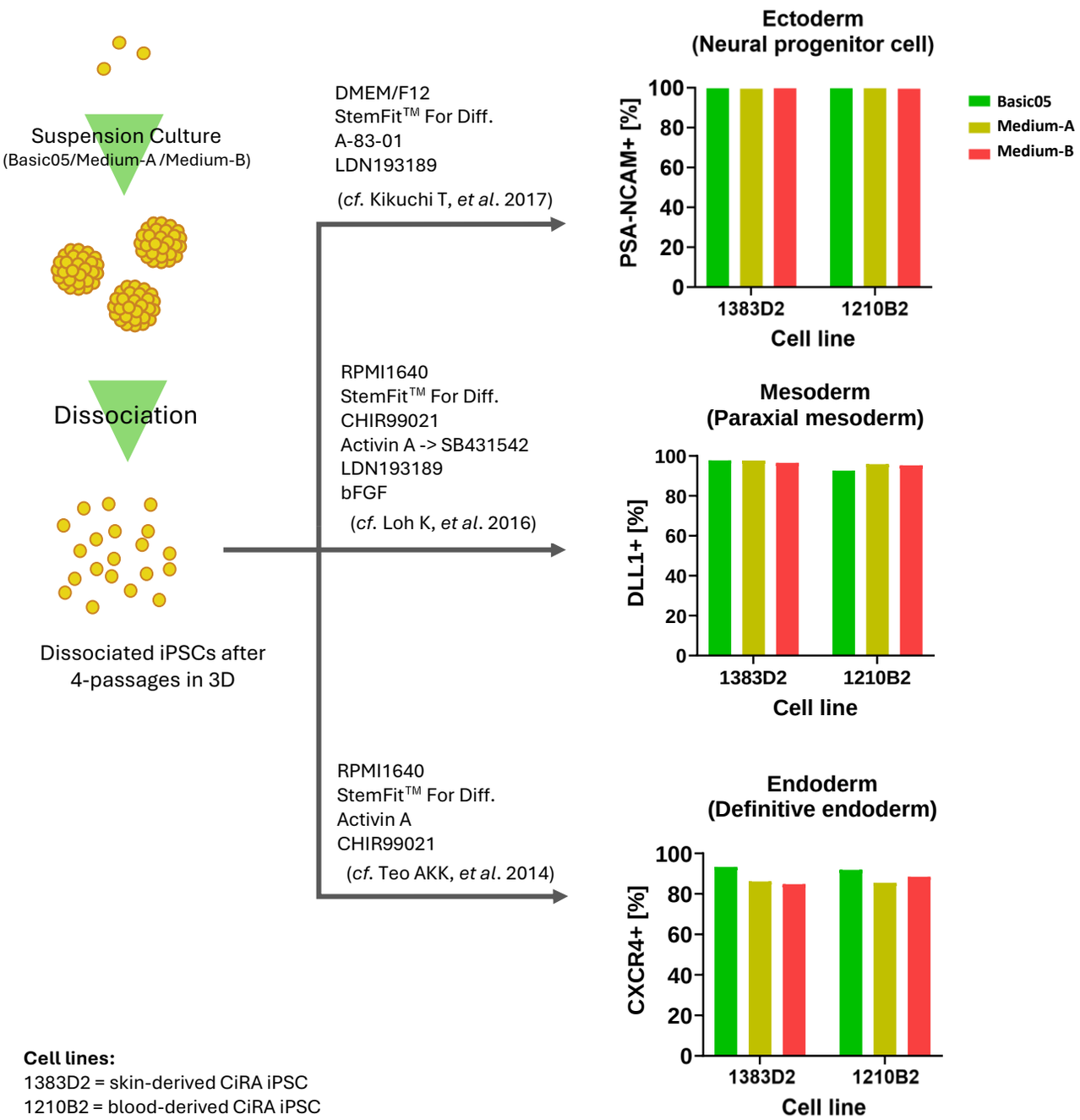


(E) Cell number over time in 3D culture



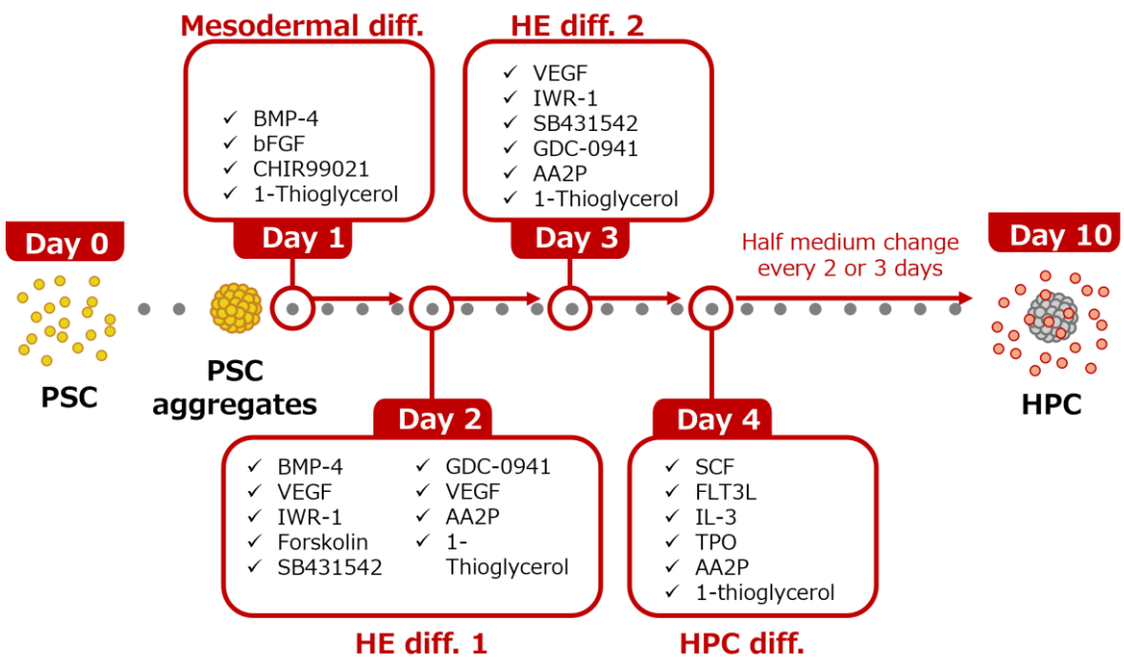
Differentiation Potential

(A) Directed differentiation to three germ layers
Basic05 supports the maintenance of differentiation potential after 3D suspension culture.
After 3D suspension culture using Basic05, iPSCs demonstrated efficient differentiation into all three germ layers, with lineage-specific marker expression confirmed across all three lineages.

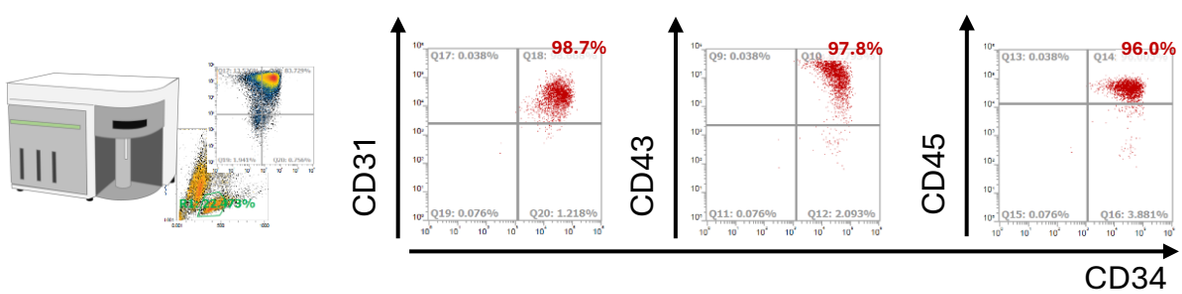


Cell lines:
1383D2 = skin-derived CiRA iPSC
1210B2 = blood-derived CiRA iPSC

(B)Directed differentiation to hematopoietic progenitor cells via PSC aggregates
Basic05 enables the direct transfer of PSC aggregates from expansion to differentiation, supporting a simplified and scalable workflow.
PSC aggregates cultured in suspension with Basic05 were directly differentiated into hematopoietic progenitor cells (HPCs).



Flow cytometry analysis



2D Performance

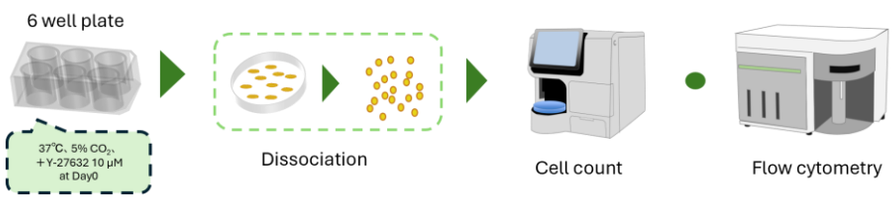
Basic05 enables a seamless transition from standard 2D culture to 3D suspension culture using a single medium, improving process development efficiency and reducing the need for re-optimization. In 2D culture, Basic05 showed strong growth and maintained pluripotency, with performance comparable to or better than conventional media.

(A) Method

bFGF was added to Basic05, and three human iPSC lines (1383D2, 201B7, and 1210B2) were cultured in 2D. Cells were cultured for four passages, and growth rate and pluripotency (OCT4-positive rate) were evaluated (7 days per passage).

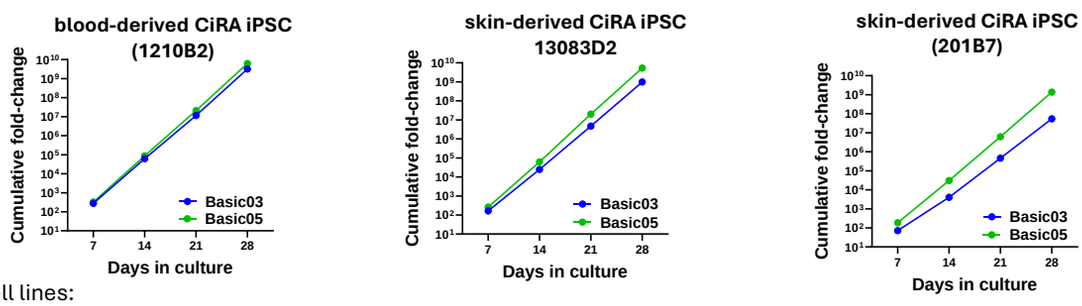
Day0	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Seeding*	Medium change (100%)	Medium change (100%)	-	-	Medium change (100%)	Medium change (100%)	Passage

*Cells were seeded at 8,000 cells for 1383D2 and 1210B2, and 13,000 cells for 201B7. iMatrix-511 was used at a coating concentration of 0.5 μg/cm^2.



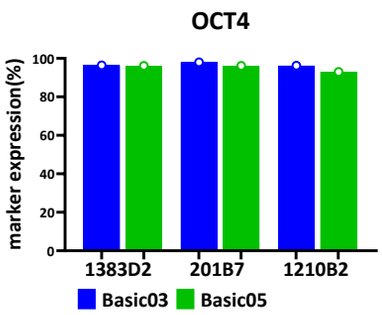
(B) Growth performance across three iPSC lines

Cumulative expansion in 2D culture was evaluated for each cell line using Basic05.
Basic05 demonstrated growth performance comparable to or better than Basic03 across all cell lines, confirming its strong performance as a 2D culture medium.



Cell lines:
13083D2 = skin-derived CiRA iPS, 1210B2 = blood-derived CiRA iPS, 201B7=skin-derived CiRA iPS

(C) OCT4-positive rate after four passages



Basic03 refers to StemFit™ Basic03, a 2D culture medium for hPSC expansion.

FAQ

Q. Is supplementation with bFGF required?

A. This medium does not contain bFGF. As bFGF signaling is required to maintain the undifferentiated state of iPS cells, supplementation with either bFGF or an FGF2 alternative (PG-011) is required. We recommend the use of PG-011.

Q. Where can I purchase PG-011?

A. PG-011 is available from PeptiGrowth Co., Ltd. For product information, please visit:
https://peptigrowth.com/products/pg-011/

Q. What types of bioreactors have been successfully used?

A. Examples include rotary shakers, stirred-tank reactors, and vertical-wheel bioreactors. Please contact us for further details.

Q. Has differentiation potential been evaluated?

A. For 3D culture differentiation, both methods have been evaluated: dissociation of spheroids prior to differentiation and direct differentiation from intact spheroids. Successful differentiation into all three germ layers has been confirmed using both approaches.

Q. We are currently using StemFit for 2D culture. Can we transition to 3D culture?

A. Yes, it is possible.

Q. Is a GMP-grade version available?

A. If you are interested in GMP-compliant products, please contact us for further information.

» Product list

StemFit™

Please request pricing!

Product	Information	
StemFit™ Basic03 (GMP)	hPSC expansion medium for clinical research and further manufacturing	Web site
StemFit™ Basic04 Complete Type (GMP)	hPSC expansion medium for basic research and clinical research one bottle composition	Web site
StemFit™ For Differentiation (GMP)	Differentiation supplement for hPSC	Web site
StemFit™ For MSC	hMSC expansion medium	Web site

StemFit Purotein™

Please request pricing!

Product	Information	
Activin A	10μg, 50μg, 1mg(0.1mg/ml)	Web site
SCF	10μg, 50μg, 1mg(0.1mg/ml)	Web site
bFGF	1mg(0.3mg/ml)	Web site
KGF	10μg, 50μg, 1mg(0.1mg/ml)	Web site
VEGF	10μg, 50μg, 1mg(0.1mg/ml)	Web site
BMP-4	10μg, 50μg, 1mg(0.1mg/ml)	Web site

» Publications and references

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Eat Well, Live Well.

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