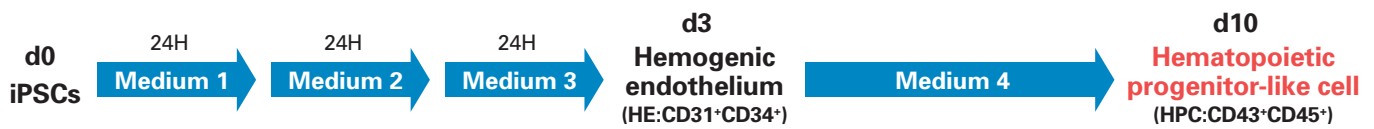


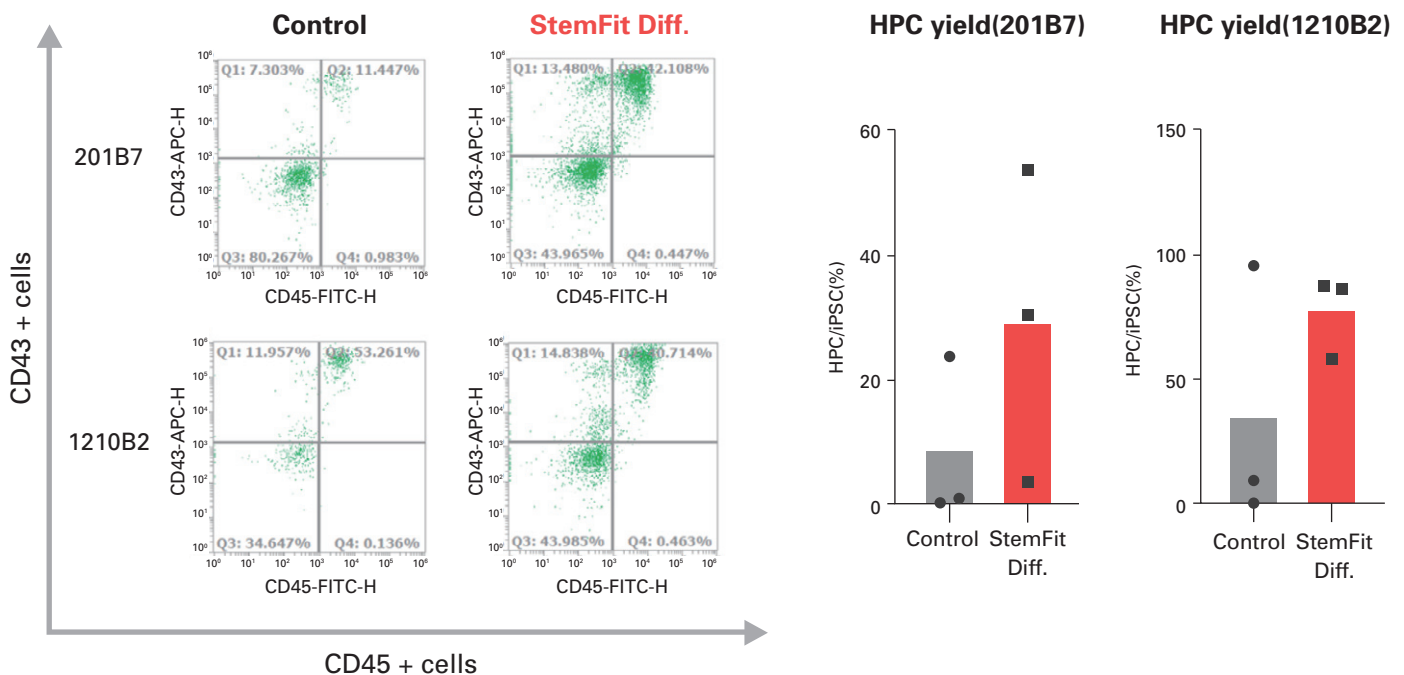
## Successful differentiation of iPSC-derived hematopoietic progenitors under chemically defined and animal origin free conditions

Animal or human derived components, such as serum derived albumin, or animal cell derived recombinant proteins, are known to carry a risk of hazardous viral contamination for cell therapy. To mitigate this, StemFit For Differentiation (StemFit Diff.) is a differentiation supplement for hPSC which is designed and manufactured under a strict animal origin free policy and is free from animal or human derived components, as referred in USP<1043> and ISO20399. With StemFit for Diff, hPSC can successfully differentiate into hematopoietic lineages under chemically defined and animal origin free conditions.



		Medium 1*	Medium 2*	Medium 3*	Medium 4**
<b>Medium</b>	Supplement	<b>20% StemFit® For Diff. or 2% Control supplement</b>			
	Basal medium	IMDM/F12			DMEM/F12
	Reagents for differentiation	40 ng/mL BMP4 30 ng/mL Activin A 20 ng/mL bFGF 6 µM CHIR99021 0.5 µM GDC-0941 400 µM MTG	40 ng/mL BMP4 10 µM SB431542 100 ng/mL VEGF 3 µM IWR-1 10 µM Forskolin 0.5 µM GDC-0941 400 µM MTG 500 µM Asc	15 ng/mL Activin A 100 ng/mL VEGF 3 µM IWR-1 0.5 µM GDC-0941 400 µM MTG 500 µM Asc	20 ng/mL SCF 10 ng/mL Flt3 ligand 5 ng/mL IL-3 20 ng/mL IL-7 10 ng/mL IL-15 1% GlutaMAX™ 400 µM MTG 250 µM Asc
<b>Other conditions</b>		iPSC(1210B2,201B7): 1x10 <sup>5</sup> cells/well Plate: 6-well plate, Scaffold: 0.5 µg/cm <sup>2</sup> VTN-N Culture: Attachment culture			HE(1210B2,201B7): 1.5x10 <sup>4</sup> cells/well Plate: PrimeSurface®(96-well, MS-9096U) Culture: low attachment culture

\*Referred from Loh MK, et al. WO2016141084 \*\* Referred from Zhu H, et al. 2019



## Differentiation into Hemogenic endothelium (HE)

Partially-confluent wells of undifferentiated hiPSCs were dissociated into single cells using Accutase and sparsely passaged on 0.5 µg/cm<sup>2</sup> iMatrix-511 or VTN-N coated 6 well plate at 1.0 – 3.0 x 10<sup>5</sup> cells in StemFit Basic03 supplemented with 80 ng/mL bFGF and 10 µM Y-27632 overnight. Seeding hiPSCs sparsely prior to differentiation was critical to prevent cellular overgrowth during differentiation, especially during long-duration differentiation. hiPSCs were allowed to plate overnight, and the following morning, were briefly washed (in IMDM/F-12) and differentiated towards Primitive Streak (PS) using Medium 1 for 24 hours. Day 1 PS was subsequently washed (IMDM/F-12) and further differentiated using Medium 2 for 24 hours. Day 2 cultures were further directed into HE by Medium 3 for 24 hours.

## Hematopoietic progenitor-like cell (HPC)

Day 3 HE was then dissociated into single cells using Accutase, counted and reaggregated on individual wells of a low-adhesion U-bottom 96-well plate (e.g., PrimeSurface® 96 well plate) with 15,000 cells in 75 µL of Medium 4 per well. 75 µL of Medium 4 was added on Day 7 (4 days after HE) and cells were further cultured for 3 days into HPC (total 10 days). If viability of HPCs is low, you can replace DMEM/F12 with IMDM as a basal medium of Medium 4.

## » Related products

Product	Information
<b>StemFit Basic03</b>	hPSC expansion medium for clinical research <a href="#">Web site</a>
<b>StemFit For Differentiation</b>	Differentiation supplement for hPSC <a href="#">Web site</a>
<b>Activin A</b>	<Non-GMP> 10µg, 50µg, 1mg(0.1mg/ml) <GMP compliant> <b>1mg(0.1mg/ml)</b> <a href="#">Web site</a>
<b>SCF</b>	<Non-GMP> 10µg, 50µg, 1mg(0.1mg/ml) <a href="#">Web site</a>

Eat Well, Live Well.



**AJINOMOTO CO., INC.** 15-1 Kyobashi 1-chome, Chuo-ku, Tokyo 104-8315

<Contact in the U.S.A> **AJINOMOTO HEALTH & NUTRITION NORTH AMERICA, INC.** Mail : [stemfit@ajiusa.com](mailto:stemfit@ajiusa.com)

<Contact in the other country> **AJINOMOTO, CO., INC.** Mail : [stemfit@asv.ajinomoto.com](mailto:stemfit@asv.ajinomoto.com)

