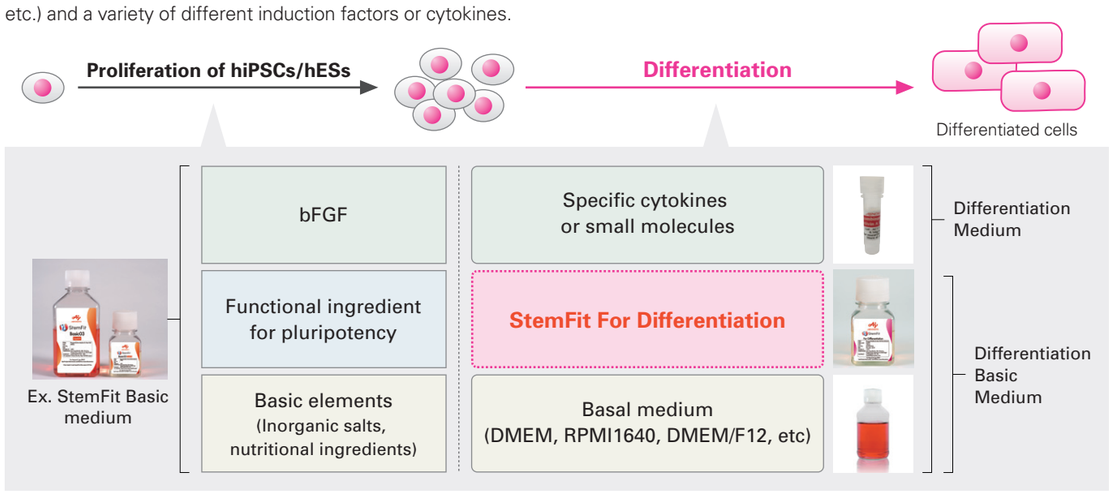
StemFit

Product Information: StemFit For Differentiation

1. Product Description

StemFit For Differentiation (Diff.) is chemically defined & animal-origin free (CD-AOF) supplement for differentiation of human ES and iPS cells. StemFit For Diff. combined with StemFit medium for the hPSCs expansion can enable clinical applications of hPSC-derived cells/tissues by providing the CD-AOF culture systems for both hPSCs expansion and differentiation.
StemFit For Diff. is provided as a **5x**concentrate and is intended to be used with basal medium (DMEM/F12、RPMI1640、DMEM etc.) and a variety of different induction factors or cytokines.



2. Materials Provided

Volume	Storage
100 ml	Store at below -20 °C

3. Prepare Differentiation Basic Medium

StemFit For Diff. is provided frozen and should be stored at below -20 °C until use. Use sterile techniques to prepare differentiation medium as follow.

1) Before use, thaw the frozen StemFit For Diff. with occasionally mixing at room temperature (15-25 °C) or overnight at 4°C. **CAUTION: Do not thaw StemFit for Diff. at 37 °C**, as it accelerates the degradation of medium
2) Add 100 mL of StemFit For Diff. (5X) to 400 mL basal medium (DMEM/F12、RPMI1640、DMEM etc.) and mix thoroughly to make differentiation basic medium. If precipitations are observed, keep the bottle at room temperature and dissolve them. *Optional: StemFit For Diff. and differentiation basic medium may be aseptically aliquoted and stored at below -20 °C. Once thawed, they may be stored at 2-8 °C for up to 2 weeks (Do not re-freeze.). We recommend storing the medium protected from light.*
3) To differentiate each lineage, induction factors or cytokines can be added as specified by differentiation protocols.
4) Before use, warm aliquots to room temperature and use immediately.

4. Contact the following department for product information:

Amino Acids Dept., AminoScience Division, AJINOMOTO CO., INC.
1-15-1 Kyobashi, Chuo-ku, Tokyo 104-8315, Japan
E-mail: **stemfit@asv.ajinomoto.com**

For Research Use Only. Not Intended For Human or Animal Diagnostic or Therapeutic Uses.

Eat Well, Live Well.
AJINOMOTO

For further information, please contact here. **stemfit@asv.ajinomoto.com**

AJINOMOTO CO., INC. AminoScience Division **https://www.ajitrade.com/stemfit/**
15-1, Kyobashi 1-Chome, Chuo-Ku, Tokyo 104-8315, Japan

Version 3 (Mar. 2022)

StemFit For Differentiation

The Next Generation Supplement

Chemically Defined & Animal-Origin Free Supplement

StemFit For Differentiation is
- chemically defined & animal component-free formulation
- enables differentiation of human ES cells and iPS cells.

High Consistency

Enables consistent cell differentiation

Free of undefined components (Serum, Human-derived component). Chemically defined composition minimizes lot-to-lot variation and enables consistent cell differentiation.

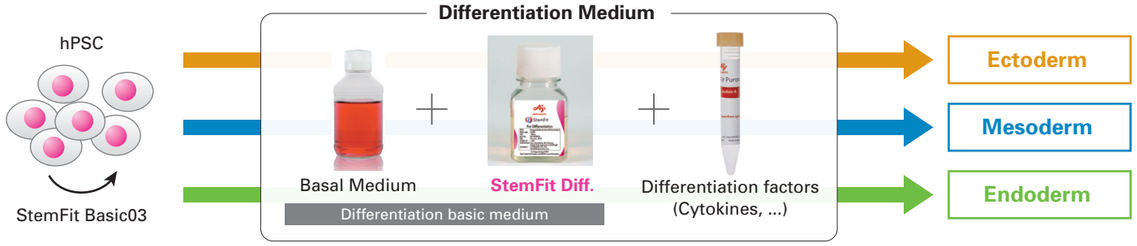
High Safety

Animal-origin free composition

Free of animal- and human-derived components. Animal-origin free composition minimizes risk of immunogenic contamination.

StemFit For Differentiation is

chemically defined & animal component-free supplement for differentiation of human ES and iPS cells to multiple lineages. It can be used with a variety of different induction factors or cytokines to support differentiation along ectoderm, mesoderm and endoderm lineages.



StemFit Diff. combined with StemFit medium for the human PSCs expansion medium, can enable clinical applications of hPSC-derived cells/tissues by providing the CD-AOF culture systems for both hPSCs expansion and differentiation. StemFit Diff. is provided as a 5×concentrate and is intended to be used with basal medium (DMEM/F12、RPMI1640、DMEM etc.) and a variety of different induction factors or cytokines.

StemFit For Differentiation can support spontaneous differentiation of hiPSCs via EB formation

Method

Attached culture (>3 weeks)

< Expansion Medium >

- StemFit Basic 03 (Ajinomoto) or
- Essential 8™ (E8, Thermo Fisher Scientific)

on 0.5 μg/cm² Vitronectin-N (Thermo Fisher Scientific)

EB formation (2 weeks)

< EB formation Medium >

- DMEM/F12+20% StemFit Diff. (StemFit Diff., Ajinomoto) or
- Essential 6 (E6, Thermo Fisher Scientific)

on ultra-low attachment microplate (Corning)

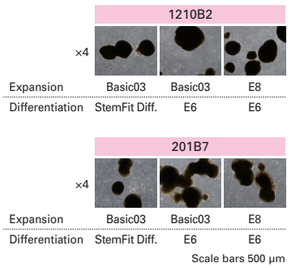
Analyze gene expression by TaqMan™ hPSC Scorecard panel (Thermo Fisher Scientific)

Combination of expantion and differentiation medium

hiPSC	Expansion	Differentiation
1210B2 (P31-36)	Basic03	StemFit Diff.
		E6
	E8	E6
201B7 (P37-42)	Basic03	StemFit Diff.
		E6
	E8	E6

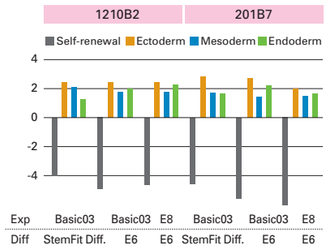
Result

Comparable EBs were formed under each condition.



Scale bars 500 μm

StemFit Diff. induced spontaneous differentiation into 3 germ layers.



StemFit For Differentiation is useful for lineage-specific differentiation in replacement of serum-free supplement

Endoderm (DE-Hepatocyte)

d0 iPSC (1231A3 on iMatrix-511)

Medium 1
StemFit Diff. basic medium
100 ng/mL Activin A
2 μM CHIR99021
100 μM Sodium Butyrate (day1~4)

d6 Definitive Endoderm (DE)

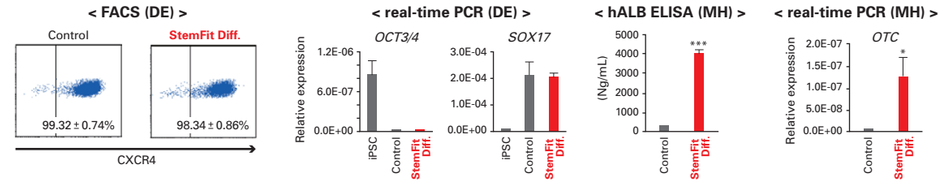
Medium 2
StemFit Diff. basic medium
0.1 mM 2-mercaptoethanol
1 mM L-glutamine
1% DMSO
1% NEAA

d13 Immature Hepatocyte (IH)

Medium 3
StemFit Diff. basic medium
100 nM Dexamethasone
10 ng/mL Oncostatin M

d21 Mature Hepatocyte (MH)

	StemFit Diff. basic medium of Medium 1	StemFit Diff. basic medium of Medium 2	StemFit Diff. basic medium of Medium 3
Control	RPMI1640 +2% B27	KnockOut DMEM +20% KSR	HCM without EGF (Lonza) +5% FBS
StemFit Diff.	RPMI1640 +20% StemFit Diff.	StemFit Basic03 (Ajinomoto)	DMEM +5% StemFit Diff.



Mesoderm (Paraxial mesoderm, Septurn trasversum mesenchyme)

d0 iPSC (1231A3 on iMatrix-511)

Medium 1
StemFit Diff. basic medium
10 μM CHIR99021
30 ng/mL Activin A
0.3 μM LDN193189
30 ng/mL bFGF

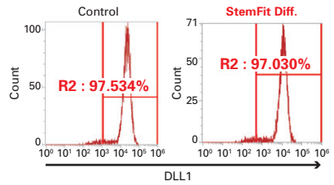
d1 Primitive streak (PS)

Medium 2
StemFit Diff. basic medium
5 μM CHIR99021
10 μM SB431542
0.3 μM LDN193189
100 ng/mL bFGF

d2 Paraxial mesoderm (PM)

Loh K, et al. 2006

	StemFit Diff. basic medium of Medium 1~2
Control	RPMI1640 +2% B27
StemFit Diff.	RPMI1640 +20% StemFit Diff.



d0 iPSC (1231A3 on iMatrix-511)

Medium 1
StemFit Diff. basic medium
25 ng/mL BMP4
8 μM CHIR99021
1% GlutaMAX

d3 Lateral plate mesoderm (LPM)

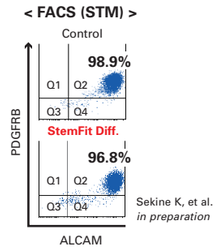
Medium 2
StemFit Diff. basic medium
10 ng/mL PDGFBB
2 ng/mL Activin A
1% GlutaMAX

Medium 3
StemFit Diff. basic medium
10 ng/mL bFGF
10 ng/mL PDGFBB
1% GlutaMAX

d7 septum transversum mesenchyme (STM)

Takebe T, et al. *Cell Reports*. 2017

	StemFit Diff. basic medium of Medium 1~2	StemFit Diff. basic medium of Medium 3
Control	DMEM/F12 +2% B27	StemPro-34 SFM
StemFit Diff.	DMEM/F12 +20% StemFit Diff.	DMEM/F12 +20% StemFit Diff.



Sekine K, et al. *in preparation*

Ectoderm (Dopaminergic neuron : DAN)

iPSC (1231A3 on iMatrix-511)

d0 – d1 Medium 1
StemFit Diff. basic medium
0.5 μM A-83-01
0.1 μM LDN193189

d1 – d4 Medium 2
StemFit Diff. basic medium
0.5 μM A-83-01
0.1 μM LDN193189
2 μM Purmorphamine

d4 – d7 Medium 3
StemFit Diff. basic medium
0.5 μM A-83-01
0.1 μM LDN193189
3 μM CHIR99021

d7 – d11 Medium 4
StemFit Diff. basic medium
0.1 μM LDN193189
3 μM CHIR99021

d11 – d28 DAN Medium 5
StemFit Diff. basic medium
5 ng/mL BDNF, 10 ng/mL GDNF
400 μM dbcAMP, 200 μM AA2M

d28 Replate for ICC **d38 DAN** Medium 5

Kikuchi T, et al. 2017

	StemFit Diff. basic medium of Medium 1~4	StemFit Diff. basic medium of Medium 5
Control	GMEM +8% KSR +1 mM sodium pyruvate +1 mM NEAA +100 μM 2-ME +4.4 μM VE acetate	Neurobasal medium +B27 Vitamin A
StemFit Diff.	DMEM/F12 +20% StemFit Diff. +4.4 μM VE acetate	DMEM +20% StemFit Diff. +0.5 mM sodeium pyruvate