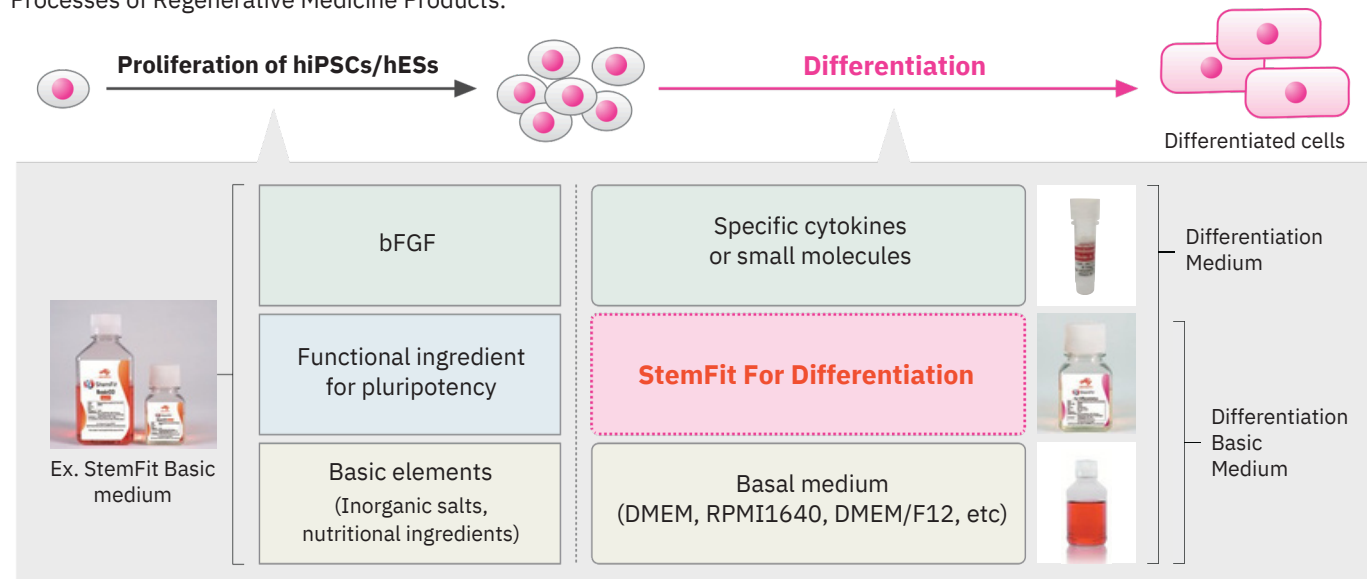


Product Information: StemFit™ For Differentiation (GMP)

1. Product Description

StemFit™ For Differentiation (Diff.) is a defined & animal-origin free (D-AOF) supplement for differentiation of human ES and iPSC cells. StemFit™ For Diff. combined with StemFit™ medium for the hPSCs expansion can enable clinical applications of hPSC-derived cells/tissues by providing the D-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.

The QMS (Quality Management System) follows the Ajinomoto System of Quality Assurance (ASQUA). Manufacturing and product release testing of this product are managed in accordance with the GMP standards Ajinomoto formulated for culture media for regenerative medicine. The GMP standards are prepared with reference to ICHQ7 and "PMDA guidance for Aseptic Manufacturing Processes of Regenerative Medicine Products."



2. Materials Provided

Product Name	StemFit™ for Differentiation	Volume	100 ml
Product Number	SF051-001	Storage	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

- 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.*
- To differentiate each lineage, induction factors or cytokines can be added as specified by differentiation protocols.
- Before use, warm aliquots to room temperature and use immediately.

StemFit™ For Differentiation is for research and further manufacturing use only and is not intended for Human or Animal diagnostic or therapeutic uses.

Eat Well, Live Well.



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

AJINOMOTO CO., INC.

15-1, Kyobashi 1-Chome, Chuo-Ku, Tokyo 104-8315, Japan

<https://www.ajitrade.com/stemfit/>

Version 4 (Dec. 2024)



StemFit™ For Differentiation

The Next Generation Supplement (GMP)



Defined & Animal-Origin Free Supplement

StemFit™ For Differentiation is Defined & animal component-free formulation - enables differentiation of human ES cells and iPSC cells.

High Consistency

Enables consistent cell differentiation

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

High Safety

Animal-origin free composition

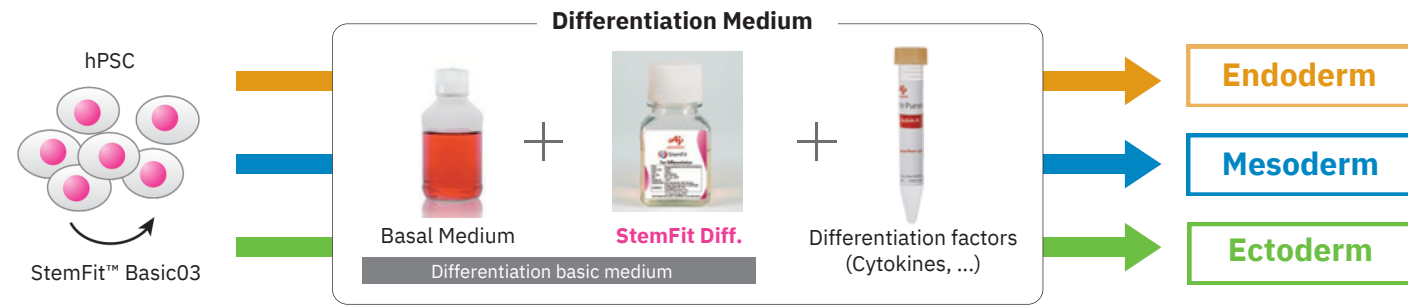
Free of animal- and human-derived components. Animal-origin free composition minimizes risk of immunogenic contamination.



StemFit™ For Differentiation is

Defined & animal component-free supplement for differentiation of human ES and iPSC 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. + StemFit™ Basic03



StemFit™ Diff. combined with StemFit™ medium for the human PSCs expansion medium, can enable clinical applications of hPSC-derived cells/tissues by providing the 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.

AJINOMOTO

Recombinant Proteins for Regenerative Medicine

<Product features>

- **Animal-origin free formulation**
- **Large batch production**
- **GMP compliant product available**
- **Frozen form (easy to use)**

Activin A

- Expression System: *E. coli*
- Product Size: 10 µg (0.1 mg/mL), 50 µg (0.1 mg/mL), 1 mg (Inquiry)

bFGF

- Expression System: *Corynebacterium glutamicum*
- Product Size: 1 mg (0.3 mg/ml)

✉ Contact us : stemfit@asv.ajinomoto.com

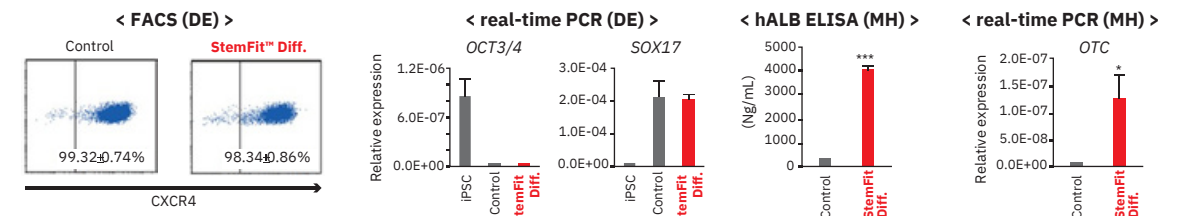
StemFit™ For Differentiation can support spontaneous differentiation of hiPSCs via EB formation

Method	Result															
<p>Attached culture (>3 weeks)</p> <p>< Expansion Medium ></p> <ul style="list-style-type: none"> • StemFit™ Basic 03 (Ajinomoto) or • Essential 8TM (E8, Thermo Fisher Scientific) <p>on 0.5 µg/cm² Vitronectin-N (Thermo Fisher Scientific)</p> <p>EB formation (2 weeks)</p> <p>< EB formation Medium ></p> <ul style="list-style-type: none"> • DMEM/F12+20% StemFit™ Diff. (StemFit™ Diff., Ajinomoto) or • Essential 6 (E6, Thermo Fisher Scientific) <p>on ultra-low attachment microplate (Corning)</p> <p>Analyze gene expression by TaqMan™ hPSC Scorecard panel (Thermo Fisher Scientific)</p> <p>Combination of expansion and differentiation medium</p> <table border="1"> <thead> <tr> <th>hiPSC</th> <th>Expansion</th> <th>Differentiation</th> </tr> </thead> <tbody> <tr> <td>1210B2 (P31-36)</td> <td>StemFit™ Basic03</td> <td>StemFit™ Diff.</td> </tr> <tr> <td></td> <td>E8</td> <td>E6</td> </tr> <tr> <td>201B7 (P37-42)</td> <td>StemFit™ Basic03</td> <td>StemFit™ Diff.</td> </tr> <tr> <td></td> <td>E8</td> <td>E6</td> </tr> </tbody> </table>	hiPSC	Expansion	Differentiation	1210B2 (P31-36)	StemFit™ Basic03	StemFit™ Diff.		E8	E6	201B7 (P37-42)	StemFit™ Basic03	StemFit™ Diff.		E8	E6	<p>Comparable EBs were formed under each condition.</p> <p>StemFit™ Diff. induced spontaneous differentiation into 3 germ layers.</p> <p>Scale bars 500 µm</p>
hiPSC	Expansion	Differentiation														
1210B2 (P31-36)	StemFit™ Basic03	StemFit™ Diff.														
	E8	E6														
201B7 (P37-42)	StemFit™ Basic03	StemFit™ Diff.														
	E8	E6														

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

Endoderm (DE-Hepatocyte)

d0	Medium 1	d6	Medium 2	d13	Medium 3	d21
iPSC (1231A3 on iMatrix-511)	StemFit™ Diff. basic medium 100 ng/mL Activin A 2 µM CHIR99021 100 µM Sodium Butyrate (day1~4)	Definitive Endoderm (DE)	StemFit™ Diff. basic medium 0.1 mM 2-mercaptoethanol 1 mM L-glutamine 1% DMSO 1% NEAA	Immature Hepatocyte (IH)	StemFit™ Diff. basic medium 100 nM Dexamethasone 10 ng/mL Oncostatin M	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, Septum transversum mesenchyme)

d0	Medium 1	d1	Medium 2	d2
iPSC (1231A3 on iMatrix-511)	StemFit™ Diff. basic medium 10 µM CHIR99021 30 ng/mL Activin A 0.3 µM LDN193189 30 ng/mL bFGF	Primitive streak (PS)	StemFit™ Diff. basic medium 5 µM CHIR99021 10 µM SB431542 0.3 µM LDN193189 100 ng/mL bFGF	Paraxial mesoderm (PM)
	StemFit™ Diff. basic medium of Medium 1~2		StemFit™ Diff. basic medium of Medium 1~2	
Control	RPMI1640 +2% B27		RPMI1640 +2% B27	
StemFit Diff.	RPMI1640 +20% StemFit™ Diff.		RPMI1640 +20% StemFit™ Diff.	

Loh K, et al. 2006

d0	Medium 1	d3	Medium 2	Medium 3	d7
iPSC (1231A3 on iMatrix-511)	StemFit™ Diff. basic medium 25 ng/mL BMP4 8 µM CHIR99021 1% GlutaMAX	Lateral plate mesoderm (LPM)	StemFit™ Diff. basic medium 10 ng/mL PDGFBB 2 ng/mL Activin A 1% GlutaMAX	StemFit™ Diff. basic medium 10 ng/mL bFGF 10 ng/mL PDGFBB 1% GlutaMAX	septum transversum mesenchyme (STM)
	StemFit™ Diff. basic medium of Medium 1~2		StemFit™ Diff. basic medium of Medium 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.	DMEM/F12 +20% StemFit™ Diff.	

Takebe T, et al. Cell Reports, 2017

Sekine K, et al. in preparation

Ectoderm (Dopaminergic neuron : DAN)

d0	d1	d4	d7	d11	d28	d38
iPSC (1231A3 on iMatrix-511)	StemFit™ Diff. basic medium 0.5 µM A-83-01 0.1 µM LDN193189	StemFit™ Diff. basic medium 0.5 µM A-83-01 0.1 µM LDN193189 2 µM Purmorphamine	StemFit™ Diff. basic medium 0.5 µM A-83-01 0.1 µM LDN193189 3 µM CHIR99021	StemFit™ Diff. basic medium 0.1 µM LDN193189 3 µM CHIR99021	StemFit Diff. basic medium 5 ng/mL BDNF, 10 ng/mL GDNF 400 µM dbcAMP, 200 µM AA2M	Replate for ICC StemFit™ Diff. basic medium of Medium 5
	StemFit™ Diff. basic medium of Medium 1~4		StemFit™ Diff. basic medium of Medium 3	StemFit™ Diff. basic medium of Medium 4	StemFit Diff. basic medium of Medium 5	StemFit™ Diff. basic medium of Medium 5
Control	DMEM/F12 +8% KSR +1 mM sodium pyruvate +1 mM NEAA +100 µM 2-ME +4.4 µM VE acetate		StemPro-34 SFM		Neurobasal medium +B27 Vitamin A	
StemFit Diff.	DMEM/F12 +20% StemFit™ Diff. +4.4 µM VE acetate		DMEM/F12 +20% StemFit™ Diff.	DMEM/F12 +20% StemFit™ Diff.	DMEM +20% StemFit™ Diff. +0.5 mM sodium pyruvate	

Kikuchi T, et al. 2017