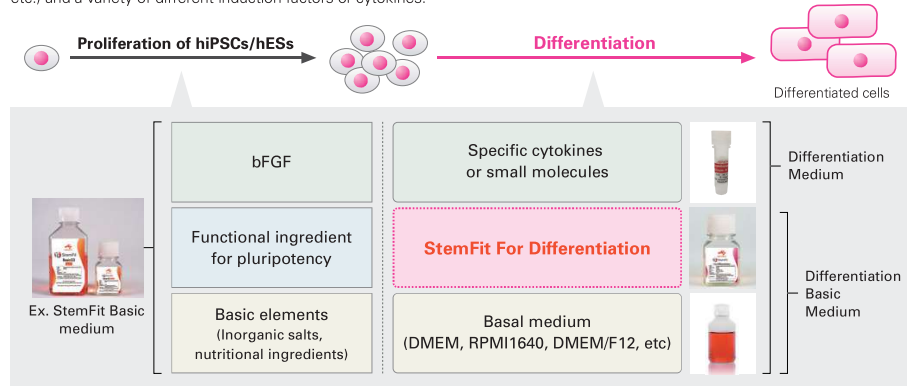


Product Information: StemFit For Differentiation

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 follows.

- 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.
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.

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

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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

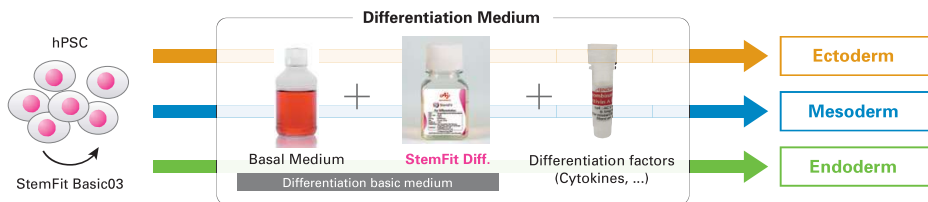
Animal-origin free composition

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 iPSCs 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 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.

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)

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 8 (E8, Thermo Fisher Scientific) on ultra-low attachment microplate (Corning)

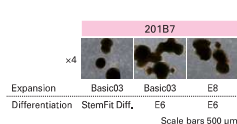
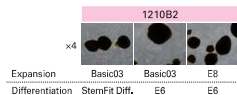
Analyze gene expression by TaqMan™ hPSC Scorecard panel (Thermo Fisher Scientific)

Combination of expansion and differentiation medium

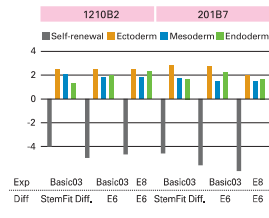
hiPSC	Expansion	Differentiation
1210B2 (P31-36)	Basic03	StemFit Diff.
	E8	E8
201B7 (P37-42)	Basic03	StemFit Diff.
	E8	E8

Result

Comparable EBs were formed under each condition.



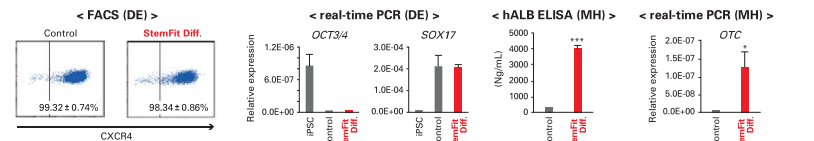
StemFit Diff. induced spontaneous differentiation into 3 germ layers.



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

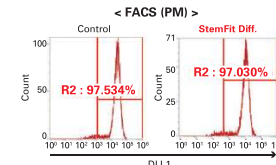
Endoderm (DE-Hepatocyte)

d0	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)
Control	RPMI1640 +2% B27	Control	KnockOut DMEM +20% KSR	Control	HCM without EGF (Lonza) +5% FBS	Control
StemFit Diff.	RPMI1640 +20% StemFit Diff.	StemFit Diff.	StemFit Basic03 (Ajinomoto)	StemFit Diff.	DMEM +5% StemFit Diff.	StemFit Diff.

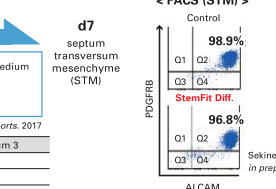


Mesoderm (Paraxial mesoderm, Septum transversum mesenchyme)

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	Promitve streak (PS)	StemFit Diff. basic medium 5 µM CHIR99021 10 µM SB431542 0.3 µM LDN193189 100 ng/mL bFGF	Paraxial mesoderm (PM)
Control	RPMI1640 +2% B27	Control	RPMI1640 +20% StemFit Diff.	Control
StemFit Diff.	RPMI1640 +20% StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.



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)
Control	DMEM/F12 +2% B27	Control	StemPro-34 SFM	Control	StemFit Diff.
StemFit Diff.	DMEM/F12 +20% StemFit Diff.	StemFit Diff.	DMEM/F12 +20% StemFit Diff.	StemFit Diff.	StemFit Diff.



Endoderm (Dopaminergic neuron : DAN)

d0	Medium 1	d1	Medium 2	d4	Medium 3	d7	Medium 4	d11	Medium 5	d28	Replate for ICC	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 0.5 µM A-83-01 0.1 µM LDN193189 3 µM CHIR99021	StemFit Diff. basic medium 0.5 µM A-83-01 0.1 µM LDN193189 3 µM CHIR99021	StemFit Diff. basic medium 5 ng/mL BDNF, 10 ng/mL GDNF 400 µM abcAMP, 200 µM AA2M	StemFit Diff. basic medium 5 ng/mL BDNF, 10 ng/mL GDNF 400 µM abcAMP, 200 µM AA2M	DAN	StemFit Diff. basic medium 5 ng/mL BDNF, 10 ng/mL GDNF 400 µM abcAMP, 200 µM AA2M	StemFit Diff. basic medium 5 ng/mL BDNF, 10 ng/mL GDNF 400 µM abcAMP, 200 µM AA2M	Replate for ICC	StemFit Diff. basic medium 5 ng/mL BDNF, 10 ng/mL GDNF 400 µM abcAMP, 200 µM AA2M
Control	DMEM/F12 +2% B27	Control	StemFit Diff.	StemFit Diff.	StemFit Diff.	Control	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	Control	StemFit Diff.
StemFit Diff.	DMEM/F12 +20% StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.	StemFit Diff.

