

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 expansion 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.

Expansion: Basic03, Basic03, E8, Basic03, Basic03, E8
 Differentiation: StemFit Diff., E6, E6, StemFit Diff., E6, E6

Scale bars 500 µm

StemFit Diff. induced spontaneous differentiation into 3 germ layers.

Expansion: Basic03, Basic03, E8, Basic03, Basic03, E8
 Differentiation: StemFit Diff., E6, E6, StemFit Diff., E6, E6

Mesoderm (Paraxial mesoderm, Septum transversum 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)

Control: RPMI1640 +2% B27
 StemFit Diff.: RPMI1640 +20% StemFit Diff.

< FACS (PM) >

DLL1

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)

Control: DMEM/F12 +2% B27
 StemFit Diff.: DMEM/F12 +20% StemFit Diff.

< FACS (STM) >

ALCAM

Sekine K, et al. in preparation

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.

Ectoderm (Dopaminergic neuron : DAN)

d0 iPSC (1231A3 on iMatrix-511)

Medium 1

- StemFit Diff. basic medium
- 0.5 µM A-83-01
- 0.1 µM LDN193189

d1

Medium 2

- StemFit Diff. basic medium
- 0.5 µM A-83-01
- 0.1 µM LDN193189
- 2 µM Purmorphamine

d4

Medium 3

- StemFit Diff. basic medium
- 0.5 µM A-83-01
- 0.1 µM LDN193189
- 3 µM CHIR99021

d7

Medium 4

- StemFit Diff. basic medium
- 0.1 µM LDN193189
- 3 µM CHIR99021

d11

Medium 5

- StemFit Diff. basic medium
- 5 ng/mL BDNF, 10 ng/mL GDNF
- 400 µM dbcAMP, 200 µM AA2M

d28 Replate for ICC DAN

Medium 5

d38 DAN

	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 sodium pyruvate

