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Maintenance of hPSC culture suitable for Quick-Tissue™ Series

For the maintenance of human pluripotent stem cells (hPSCs) in StemFit® Basic02 Conditions

This protocol works for both human embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)

For the following products:

- Ajinomoto StemFit® Basic02 medium (Elixirgen Scientific Cat#EXGS-ASB02)
- Nippi iMatrix-511 silk (Elixirgen Scientific Cat#EXGS-NI511S)

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I. Introduction

The Quick-Tissue™ Differentiation kits give their best differentiation results when the source hPSCs are routinely maintained in StemFit® Basic02 (StemFit®) culture medium.

NOTE: EXPERIMENT SCALE

In this protocol, instructions are described for a 35-mm dish or one well of a 6-well plate. For other well sizes, multiply the protocol's recipe by the ratio between well sizes.

II. Materials Required

- Ajinomoto StemFit® Basic02 medium (Elixirgen Scientific Cat#EXGS-ASB02)
- Nippi iMatrix-511 silk (Elixirgen Scientific Cat#EXGS-NI511S)
- bFGF (i.e. FGF2) growth factor (10 µg/ml, sterile-filtered)
- ROCK inhibitor
- TrypLE Select (Life Tech, Cat#12563-029)
- Phosphate-buffered saline (PBS without Ca^{++} Mg^{++})
- 0.5 mM EDTA in PBS
- STEM-CELLBANKER (Amsbio, Cat#11897)

III. Pre-Protocol Preparation

Complete StemFit® Medium

1. Thaw StemFit® Basic02 medium Liquid A (400 ml) and Liquid B (100 ml) at 4°C overnight.
2. Add Liquid B into Liquid A. Mix them well. We recommend aliquoting this medium into 45 ml aliquots in 50 ml Conical Centrifuge Tubes (e.g., Falcon™, Corning®) and storing the aliquots at -80°C.
3. Thaw a 45-ml aliquot at 4°C overnight.
4. Add 450 µl 10 µg/ml bFGF to 45 ml StemFit® Basic02 (A+B) before use (the final concentration of bFGF is 100 ng/ml). This medium is referred to as Complete StemFit® Medium and is stable for up to 2 weeks at 4°C.

iMatrix-511 silk Coated Plates

1. Take 12 ml ice-cold PBS into a tube and add 28.5 µl iMatrix-511 silk (0.5 mg/ml). Mix them well. This mixture is stable for up to 2 weeks at 4°C.
2. Add 2 ml diluted iMatrix to one well of a 6-well plate or a 35-mm culture dish.
3. Incubate the plate or dish at 37°C, 5% CO_2 for 2 hours (or 4°C overnight.)
4. Aspirate the supernatant from each well or dish and add 2 ml PBS to it.
5. Incubate the plate or dish at 37°C, 5% CO_2 until user's hPSCs are ready for plating or up to 1 hour.

10 mM Rock Inhibitor

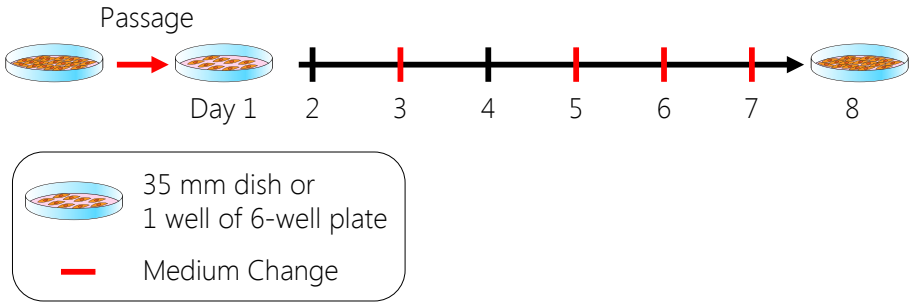
1. Prepare ROCK inhibitor stocks at a concentration of 10 mM in molecular-biology-grade DMSO. The stock is stable for up to 2 weeks at 4°C.
2. We recommend aliquoting 10 mM ROCK Inhibitor into 100 µl aliquots and storing the aliquots at -20°C.

0.5x TrypLE Select

1. Dilute TrypLE-Select (1x) with 0.5 mM EDTA in PBS at a ratio of 1:1. The mixture is referred to as 0.5x TrypLE Select and is stable for up to 2 months at 4°C.

   Note: A trypsin inhibitor is not required when using TrypLE Select as dilution will inactivate TrypLE Select mostly. However, we still recommend minimizing carryovers of TrypLE Select in culture.

IV. Protocol



NOTE: CELL CONFLUENCY

- hPSC culture is best for passaging when the cell confluency reaches 80-90%. However, when the growing colonies start to touch each other, consider harvesting them even if the cell confluency is below 80%. Otherwise, cells begin to differentiate.

Day 1-2 - Passaging

1. Warm Complete StemFit® Medium and 0.5x TrypLE Select for 20-30 minutes to room temperature.
2. Take 4 ml Complete StemFit® Medium into a tube and add 4 µl 10 mM ROCK Inhibitor to it. Mix them well.
3. Aspirate old medium from the culture and add 1.5 ml Complete StemFit® Medium with 10 µM ROCK inhibitor to it.
4. Incubate the culture at 37°C, 5% CO_2 for 1 hour. This treatment enhances the survival of hPSCs.
5. Aspirate the old medium from the culture and add 2 ml PBS to it.
6. Aspirate PBS from the culture and add 300 µl 0.5x TrypLE Select to it.
7. Incubate the culture at 37°C, 5% CO_2 for up to 10 minutes.
8. Carefully aspirate 0.5x TrypLE Select from the culture using a P1000 pipettor and add 1 ml PBS to it.
9. Gently rock the plate or dish 3 times.
10. Carefully pipet out PBS using a P1000 pipettor and add 1 ml Complete StemFit® Medium with 10 µM ROCK inhibitor.
11. Disperse the medium over the well bottom surface by pipetting 8-15 times to detach cells.
12. Collect the cell suspension in a tube.
13. Count cells to determine the volume of cell suspension needed to plate 1 x 10^4 cells.
14. Take out the determined volume of the cell suspension from the previous step in a tube and bring up the volume to 1.5 ml with with Complete StemFit® Medium with 10 µM ROCK inhibitor.
15. Aspirate PBS from a new iMatrix-511 silk-coated well and add 1.5 ml cell suspension to it.
16. Rest of the cells can be used for assays or frozen down using STEM-CELLBANKER (2x10^5 cells per vial).
17. Incubate the culture at 37°C, 5% CO_2 for 1 hour.
18. Observe the culture under a microscope and make sure that cells are evenly distributed in the well or dish.
19. Incubate the culture at 37°C, 5% CO_2 for 2 days.

NOTE: CONFLUENCY AND FEEDING FREQUENCY

- Our data indicate that a culture prepared using 1.0 x 10^4 cells reaches 80-90% confluency and produces about 2-3 x 10^6 cells in 7-8 days.
- When 1.0 x 10^4 cells are plated on each well or dish, feed cultures every 2 days for the first 4 days.
- When more than 1.0 x 10^4 cells are plated on each well or dish, feed cultures with 2-4 ml medium every day. The culture reaches 80-90% confluency in 3-4 days.

Day 3-4 - Feeding

1. Warm Complete StemFit® Medium for 20-30 minutes to room temperature.
2. Aspirate the old medium from each well or dish and add 1.5 ml Complete StemFit® Medium to it.
3. Incubate the culture at 37°C, 5% CO_2 for 2 days.

Day 5-7 - Feeding

1. Warm Complete StemFit® Medium for 20-30 minutes to room temperature.
2. Aspirate the old medium from each well or dish and add 1.5 ml Complete StemFit® Medium to it.
3. Incubate the culture at 37°C, 5% CO_2 overnight.
4. Repeat steps 1-3 until Day 8.

Day 8 - Ready for Quick-Tissue™ Differentiation Kits or Continuous Culture

The culture should reach 80-90% confluency and is ready for any Quick-Tissue™ kit or starting new cultures. For starting new cultures, follow the instruction on Day 1.

V. References

1. Nakagawa M, Taniguchi Y, Senda S, Takizawa N, & Ichisaka T (2014). A novel efficient feeder-free system for the derivation of human induced pluripotent stem cells. Scientific Reports, 8: 1–7.
2. Akiyama T, Wakabayashi S, Soma A, Sato S, Nakatake Y, Oda M, Murakami M, Sakota M, Chikaza-wa-Nohtomi N, Ko SB, Ko MS (2016). Transient ectopic expression of the histone demethylase JMJD3 accelerates the differentiation of human pluripotent stem cells. Development, 143: 3674-3685.
3. Goparaju SK, Kohda K, Ibata K, Soma A, Nakatake Y, Akiyama T, Wakabayashi S, Matsushita M, Sakota M, Kimura H, Yuzaki M, Ko SB & Ko MS (2017). Rapid differentiation of human pluripotent stem cells into functional neurons by mRNAs encoding transcription factors. Scientific Reports 7, 42367.
4. Morizane R, Bonventre JV (2017). Generation of nephron progenitor cells and kidney organoids from human pluripotent stem cells. Nat Protoc., 12: 195-207.

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Category	Product	Request Quote (Catalog number)		
		SeV Complete Kit	mRNA Complete Kit	Maintenance Medium
Quick-Tissue™ Series Differentiation Kit (mainly for 4 wells of 24-well plate)	Quick-Endothelium™ Vascular with Optional Drug Selection		$399 (EXGS-QEV)	$229 (EXGS-QEVM)
	Quick-Trilineage™ Differentiation Kit*	$549 (EXGS-Q3D)		
	Quick-Neuron™ Mixed	$349 (EXGS-QNMSV)		$129 (EXGS-QNMM)
	Quick-Neuron™ Cholinergic	$349 (EXGS-QNCSV)	$299 (EXGS-QNC)	$129 (EXGS-QNCM)
		$999 (EXGS-QNCSV96)**		
	Quick-Neuron™ Dopaminergic	$399 (EXGS-QNDSV)	$399 (EXGS-QND)	$169 (EXGS-QNDM)
	Quick-Neuron™ GABAergic	$399 (EXGS-QNGSV)	$399 (EXGS-QNG)	$149 (EXGS-QNGM)
	Quick-Muscle™ Skeletal	$349 (EXGS-QMSSV)		$129 (EXGS-QMSM)
	Quick-Hepatocyte™	$499 (EXGS-QHSV)		$149 (EXGS-QHM)
	Quick-miniBrain™	$999 (EXGS-QMBSVMR)		

15% off for 3 or more kit purchases per order

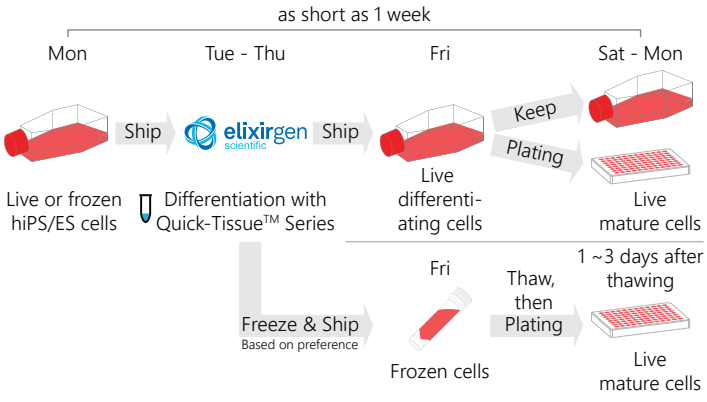
* This kit provides 2 wells per each tissue (total 6 wells of 24-well plate)

** This kit is for 48 wells of a 96-well microplate format

Category	Product	Size	Request Quote (Catalog number)
Quick-Tissue™ Series Differentiation Support	Quick-Tissue™ Mesendoderm Booster	8 wells of 24-well plate	$99 (EXGS-QTMB)
	Quick-Tissue™ Adaptation Kit	a 35-mm dish or 1 well of 6-well plate	$169 (EXGS-QTA1)
	New product coming soon!		
Reagents for Maintaining Undifferentiated Stem Cells	Ajinomoto StemFit® Basic02	500 mL	Ask (EXGS-ASB02)
	Nippi iMatrix-511 silk	175 µg x 6 tubes	Ask (EXGS-NI511S)
	Nippi iMatrix-511	175 µg x 6 tubes	$690 (EXGS-NI511)

15% off for 3 or more kit purchases per order

Quick-Tissue™ Stem Cell Differentiation Services



Elixirgen Scientific provides pluripotent stem cell differentiation services with the world's fastest turnaround time. Customers can simply ship live iPS/ES cells in a T-25 flask and will receive live or fronzen cells in a week (express service) or two weeks (regular service). Contact services@elixirgenscientific.com for more details to customize for your project.
Currently Elixirgen Scientific offers all tissue types from kits for cell differentiation services. More tissue types are coming soon!