I. Introduction

StemFit® Basic04 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

- StemFit® Basic04 medium (amsbio, Cat#SFB-504)
- iMatrix-511 (Cat# AMS.892)
- bFGF (i.e. FGF2) growth factor (10 µg/ml, sterile-filtered) (amsbio, Cat#AMS-480)
- 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#11890 and Cat#11897)

III. Pre-Protocol Preparation

Complete StemFit® Medium

1. Thaw StemFit® at 4°C overnight. Before use, thaw at room temperature (15-25ºC). CAUTION: Do not thaw at 37°C, as this accelerates the degradation of the medium components.

2. Upon thawing, add bFGF at a concentration of 10 ng/ml.

Note: For new users and cell lines we recommend starting with a concentration of bFGF (e.g. 40 - 80 ng/ml) according to suit your cell line if your cells differentiate.

CAUTION: **Thawed StemFit® Basic04 medium may be stored at 2 - 8°C for up to two weeks.**

CAUTION: **We recommend storing the medium in the dark**

3. **StemFit® Basic04** medium can be aseptically into 45 ml aliquots in 50 ml Conical Centrifuge Tubes (e.g., Falcon™, Corning®), store aliquots at -20°C. Before use, thaw an aliquot in the refrigerator overnight. Warm aliquots to room temperature and use immediately.

CAUTION: **Do not heat the thawed medium to 37°C.**

Coated Plates with ECM

NOTE: CHOICE OF MATRICE

For maximum cloning efficiency, we recommend using recombinant laminin-511 E8 available from amsbio. StemFit® Basic04 has been proven to perform with alternative matrices such as Matrigel, vitronectin, Geltrex and laminin-521

1. Take 12 ml ice-cold PBS into a tube and add 28.5 µl iMatrix-511 (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 10µM 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 StemFit® with 10 µM ROCK inhibitor.

15. Aspirate PBS from a new iMatrix-511 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

Data indicates 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.

CAUTION: **If the color of the medium turns orange or yellow, it should be changed every day.**

CAUTION: **Do not allow cells to become confluent.**

V. FAQs & Troubleshooting

1) What are the benefits of single cell culture? / Why is single cell culture recommended?

   High fold expansion rate (~100x expansion / weekly passage)

   Reproducible and manageable culture by controlling the numbers of seeded cells

   Cost-effective culture with lower medium volume and less frequent medium changes

   Produce an iPSC colony derived from single cells. (essential for genome editing)

2) Can I use StemFit® for clump culture?

   Yes, but we recommend making a small clump and seeding at a low cell density.

3) Cells do not grow well.

   Adjust the bFGF concentration (e.g. 40 - 80 ng/ml) according to your cell line

   Try a higher seeding density (e.g. > 1.0 x 10^5 cells per well (6-well plate))

Distribute the cells evenly upon passage

Culture in Y-27632-containing medium for more than 24 hours

Make sure that the medium was thawed within 2 weeks and has not been heated to 37ºC

VI. 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, Chikazawa-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.

Version 1 (May, 2019)

AMSBIO| www.amsbio.com | info@amsbio.com

UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85