Instruction Manual
Maintenance and expansion of human ES/iPS cells with StemFit medium

1. Materials Required

StemFit medium (e.g. StemFit Basic02, Basic03, Basic04, Basic04 Complete Type)

Cell dissociation reagents (e.g. Accutase™, TrypLE™ Select)

Extracellular Matrix (e.g. LDEV-free hESC qualified Geltrex™)

Human bFGF (Not required for Basic04 Complete Type)

10 mM Y-27632

PBS (-)

2. Media Preparation

StemFit medium is provided frozen. StemFit medium should be stored at below -20°C until use. Use sterile techniques to prepare StemFit medium.

1) Thaw frozen StemFit medium with occasional mixing at room temperature (15-25°C) or in a refrigerator (2-8°C). Avoid prolonged exposure to light.
   Note: Do not thaw at 37°C, as it accelerates the degradation of the medium ingredients.
2) Aseptically mix medium components by adding the full volume of “Liquid B” to “Liquid A”. Mix thoroughly.
   Note: Basic04 or Basic04 Complete Type does not require this step.
3) Add bFGF at a final concentration of 80 ng/mL. Mix thoroughly.
   Note: Do not warm bFGF solution.
   Note: It is recommended to adjust the concentration of bFGF accordingly to suit your cell line.
   Note**:** Basic04 Complete Type does not require this step since it contains bFGF.
4) Optionally, the medium can be stored as aliquots at -20°C until the expiration date. Avoid repeated freeze-thaw cycles of the aliquots. Before use, thaw an aliquot in the refrigerator (2-8°C) overnight.
5) Store the thawed medium in the refrigerator.
   Note: Thawed StemFit medium may be stored at 2-8°C for up to two weeks.
   Note: It is recommended to store the medium in the dark.
6) Before use, warm aliquots to room temperature and use immediately.
   Note: Do not warm the thawed medium to 37°C.

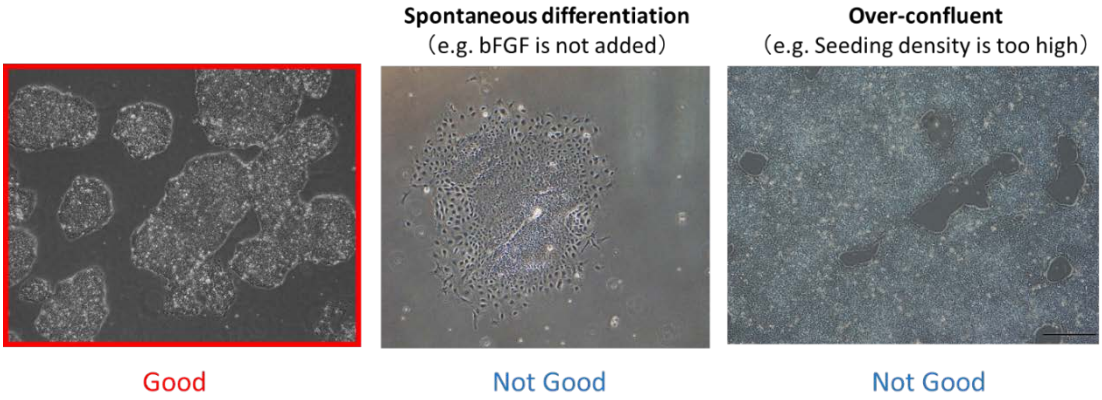
3. Passage Protocol (6-well plate)

- **Culture plate coating:**

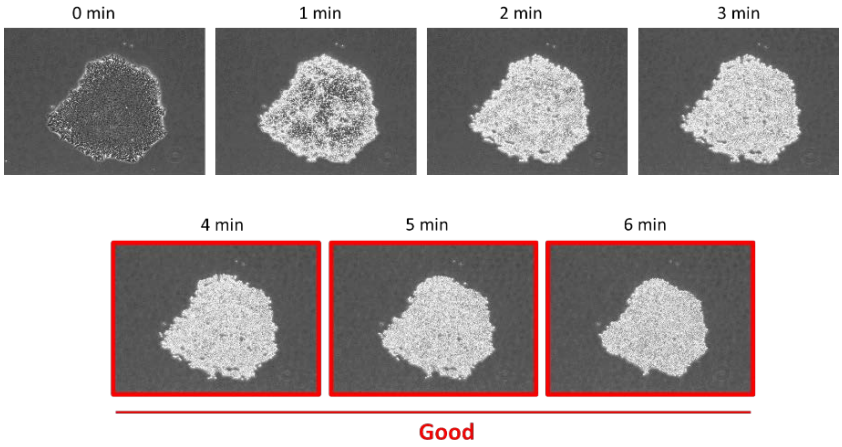
Add LDEV-free hESC-qualified Geltrex™ to cold DMEM/F-12 at a 1:100 ratio and mix well immediately. Add 1 mL of the Geltrex mixture to one well of a 6-well plate. Incubate at 37°C for at least 1 hour.
Note: You can use other matrices such as Matrigel, vitronectin, laminin-521, laminin-511 or laminin-511E8.

■ **Passage (Also see the technical tips: Key points for successful single-cell passage)**

1) Prepare "passage medium", StemFit medium containing 10 μM of the ROCK inhibitor Y-27632.
   For example, add 4 μL of 10 mM Y-27632 to 4 mL of StemFit medium.
2) Take out the culture plate from the incubator and observe the cells under a microscope. Observe cell density, colony size, and cell morphology to confirm that the cells are suitable for passage.



3) Remove the medium and wash cells with 1 mL of PBS.
4) Add 500 μL of cell dissociation reagents (e.g. Accutase™, TrypLE™ Select).
5) Incubate at 37 °C for 5-10 minutes.
   Note: The dissociation condition needs to be optimized for each cell culture in your experiment.
   Note: The incubation time is approximately up to 15 minutes.



6) Detach cells by gentle pipetting up and down.
7) Transfer cells into a new polypropylene tube. Add 500 μL of passage medium prepared in step 1) to the tube containing the cell suspension.
8) Centrifuge at 300×*g* at room temperature for 5 minutes.
9) After centrifugation, aspirate the supernatant and add 1 mL of passage medium.
10) Gently pipette cells up and down approximately 10 times to create a single-cell suspension.
    Note: Pipette gently to avoid foaming.
11) Take a portion of the cell suspension for counting. Determine the cell concentration.
12) Remove coating solution from a well of prepared matrix-coated plate.
13) Add 1.5 mL of passage medium prepared in step 1) to the well immediately.
    Note: Solutions should be replaced quickly, not to allow the coated wells to dry out.
14) Seed 10,000-50,000 cells to a well of 6-well plate. 50,000 cells are recommended at first.

Note: Before Seeding, mix cell suspension by gently pipetting up and down.

Note: Seeding cell number should be optimized for each cell line.

15) Immediately, shake the plate to distribute cells.

Note: To avoid uneven distribution on the bottom, plate shaking should be performed immediately after seeding.

16) Incubate cells at 37°C, 5% CO_2 for more than 24 hours.

17) Exchange medium the next day. Remove the passage medium and gently add 1.5 mL of StemFit medium without Y-27632.

Note: Passage medium should be replaced after more than 24 hours.

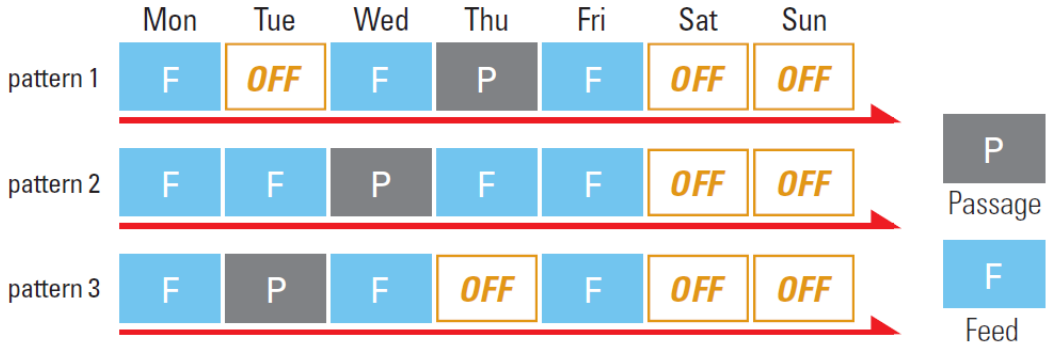
18) Passage the cells every 7 days.

Note: You can culture hPSCs without weekend medium changing. See the following passage schedule examples.

Note: If the color of the medium becomes orange or yellow, it should be changed every day.

Note: Do not allow cells to become confluent.

StemFit Passage Schedule Examples (Weekend-free feeding)



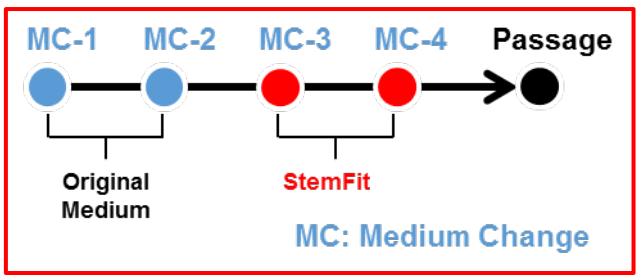
Reccomended volume of culture medium

	6-well	12-well	24-well	96-well
Approximate area (cm^2)	**9.6**	**4.0**	**2.0**	**0.33**
Volume of medium (mL)	**1.5**	**1**	**0.5**	**0.1**

4. Transfer from other culture systems

- To transfer cells from other culture systems to the StemFit system, it is recommended to passage with the original culture system, then to switch the culture medium to StemFit supplemented with bFGF 2-3 days prior to the next passage.
- Seeding the cells at a higher density (>1.0 x 10^5 cells per well (6-well plate)) may be helpful for the first few passages.

Transition Schedule Example



5. Reference

Morizane, R. & Bonventre, J. V. Generation of nephron progenitor cells and kidney organoids from human pluripotent stem cells. *Nat Protoc.* 2017 Jan;12(1):195-207.

6. FAQs & Troubleshooting

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

- High fold expansion rate (~100× 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 it is recommended to make a small clump and seeding at a low cell density.

3) Which bFGF can be used ?

- Any commercially available bFGF have been confirmed to work. Ajinomoto provides high quality, animal-origin free bFGF (Item code: SP-FGF2-G-001MG).

4) 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 a well of 6-well plate).
- Distribute the cells evenly upon passage.
- Culture in Y-27632-containing medium for more than 24 hours after passaging.
- Make sure that the medium was thawed within 2 weeks and has not been heated to 37°C.
- Detached cells with low viability may not grow well.

7. Contact information

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