Instruction Manual for StemFit with iMatrix-511*

Maintenance and expansion of human ES/iPS cells with StemFit medium and iMatrix-511

*This manual is optimized for iMatrix-511. Please see other instruction manuals for other extracellular matrices.

1. Materials Required

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

0.5x TrypLE™ select CTS*

iMatrix-511

Human bFGF (Not required for Basic04 Complete Type)

10 mM Y-27632

PBS (-)

* Preparation of 0.5x TrypLE™ select (10 mL)

1) Add 5 mL of TrypLE™ select CTS (ThermoFisher, A12859-01) into 50-mL tube.
2) Add 5mL of PBS(-).
3) Add 5 μL of 0.5 M EDTA pH8.0 solution (ThermoFisher, 15575-038).
4) Mix thoroughly. Store at R.T.

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℃). 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 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℃ until the expiration date. Do not re-freeze thawed aliquots. Before use, thaw an aliquot in the refrigerator (2-8ºC) 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.

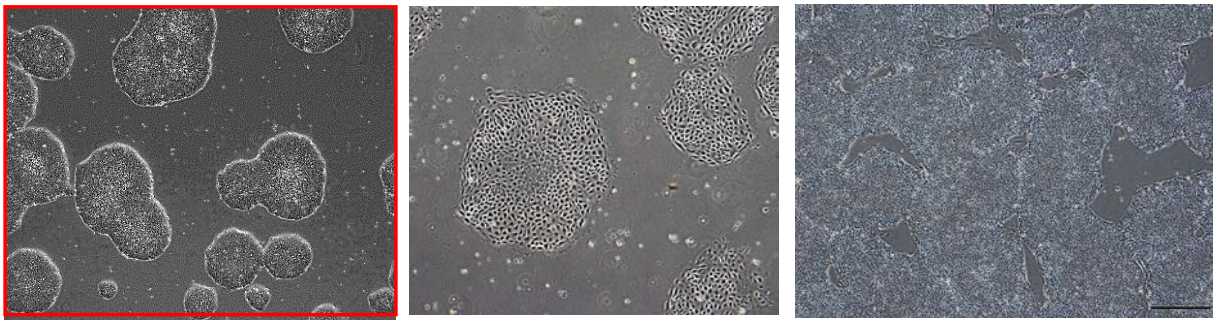
3. Passage Protocol (6-well plate)

■ **Culture plate coating:**

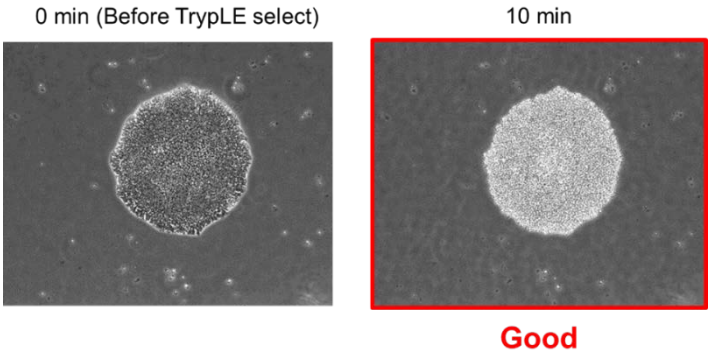
1) Add 9.6 μL (4.8 μg) of iMatrix-511 to 1.5 mL of PBS in a new polypropylene tube.
2) Add 1.5 mL of coating solution prepared in step 1) into a well of the 6-well plate. (coating: 0.5 μg/cm^2).
3) Incubate at 37°C for 1 hour or overnight at 4°C.

■ **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 300 μL of 0.5x TrypLE Select.
5) Incubate at 37°C for 10 minutes.
   Note: Incubation time may vary depending on the matrix or cell types, limiting the upper limit to 20 minutes.
   Note: Check the cell morphology in the entire field of wells with a microscope at the beginning, in the middle, and at the end of dissociation step.



6) Remove TrypLE Select and wash gently with 1 mL of PBS.
7) Add 1 mL of passage medium prepared in step 1).
8) Detach cells immediately by gentle pipetting up and down approximately 10 times.
   Note: Pipette gently to avoid foaming.

Note: If cells are not detached from the plate, cell scraper can be used for detachment.

9) Transfer cells into a new polypropylene tube.
10) Take a portion of the cell suspension for counting. Determine the cell concentration.
11) Remove coating solution from a well of prepared iMatrix-511-coated plate.
12) 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.
13) Seed 10,000-20,000 cells to a well of 6-well plate. 20,000 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.
14) Immediately, shake the plate to distribute cells.
    Note: To avoid uneven distribution on the bottom, plate shaking should be performed immediately after seeding.
15) Incubate cells at 37℃, 5% CO_2 for more than 24 hours.
16) 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.
17) 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.

■ **Option: Uncoat Passage Protocol (6-well plate without coating; skip culture plate coating step)**

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 300 μL of 0.5x TrypLE Select.
5) Incubate at 37°C for 10 minutes.
   Note: Incubation time may vary depending on the matrix or cell types, limiting the upper limit to 20 minutes.
   Note: Check the cell morphology in the entire field of wells with a microscope at the beginning, in the middle, and at the end of dissociation step.
6) Remove TrypLE Select and wash gently with 1 mL of PBS.
7) Add 1 mL of passage medium prepared in step 1). Blow out medium directly onto the colonies to detach cells.
8) Detach cells by gentle pipetting up and down approximately 10 times.
   Note: Pipette gently to avoid foaming.
   Note: If cells are not detached from the plate, cell scraper can be used for detachment.
9) Transfer cells into a new polypropylene tube.
10) Take a portion of the cell suspension for counting. Determine the cell concentration.
11) Add 5 μL of iMatrix-511 into 1.5 mL of passage medium prepared in step 1) in a new polypropylene tube.
12) Add 10,000-20,000 cells into the tube prepared in step 11).
13) Add cell suspension prepared in step 12) to a well. (Final concentrations: 0.25 μg/cm^2 iMatrix-511, 10,000-20,000 cells/well, 1.5 mL/well).

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

15) 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.

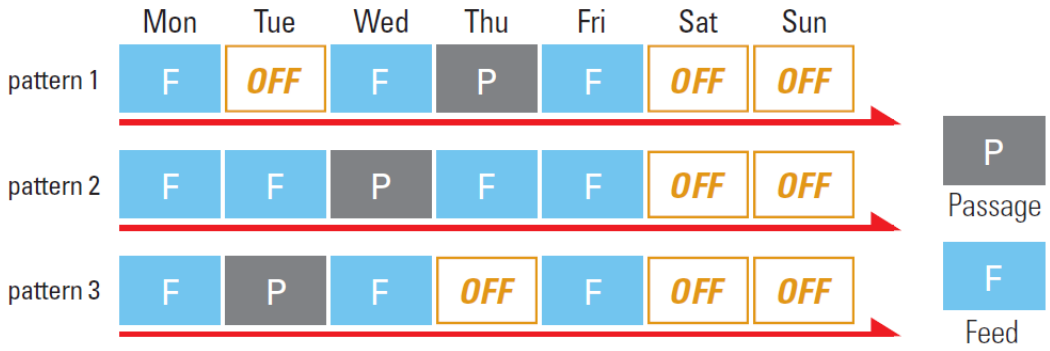
16) Passage the cells every 7 days.

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)



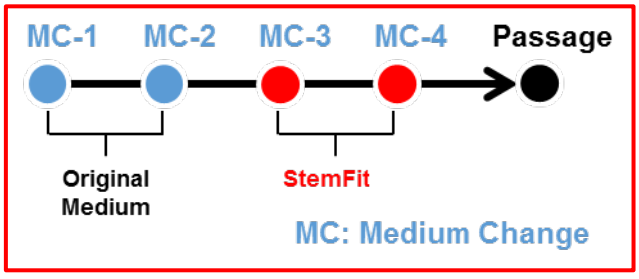
Reccomended volume of culture medium

	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**
Volume of iMatrix-511 (μL)	**5**	**2**	**1**	**0.33**

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. 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.
- Uncoated method cannot be applied high density-seeding. (Cells poorly attach to the wells).

5) Cells do not attach to the wells.

- Precisely count cell number in the cell suspension, calculate volume to seed.
- Cryopreserved cells cannot be seeded by the uncoated method since they are usually seeded in high density.

6. Contact information

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