Instruction Manual: StemFit®Basic03
For maintenance and expansion of human ES/iPS cells

1. Materials Required

StemFit®Basic03 ,

Cell dissociation reagents (e.g. Accutase),

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

Human bFGF,

Y-27632,

PBS (-)

2. Media Preparation

StemFit®Basic03 (Basic03) is provided frozen as a 2-component set containing “Liquid A” and “Liquid B”, and can be stored at below -20°C until use. Use sterile techniques to prepare Basic03 medium.

1) Before use, thaw frozen “Liquid A” and “Liquid B” with occasional mixing at room temperature (15-25°C).
   ***CAUTION:* Do not thaw “Liquid B” 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.
3) Upon thawing, StemFit®Basic03 medium can be aseptically aliquoted and stored at below -20°C. Before use, thaw an aliquot in the refrigerator overnight.
4) **Add bFGF at a concentration of 40 ng/ml**.
   Note: We recommend adjusting the concentration of bFGF according to suit your cell line.
5) Store the thawed medium in the refrigerator.
   ***CAUTION:* Thawed StemFit®Basic03 medium may be stored at 2 - 8°C for up to two weeks.**
   ***CAUTION:* We recommend storing the medium in the dark.**
6) Before use, warm aliquots to room temperature and use immediately.
   ***CAUTION:* Do not heat the thawed medium to 37°C.**

3. Passage Protocol (6-well plate; Also see our technical tips: Key points for successful single-cell passage)

1) Culture vessel 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 six-well plate. Incubate at 37°C for at least 1 h.
   Note: You can use other matrices such as Matrigel, vitronectin, laminin-521, laminin-511 or laminin-511E8.
2) Cell passage: Aspirate the medium and wash once with 2 ml of PBS/well.
3) Aspirate the PBS and add 500 µl of Accutase. Incubate at 37°C for 10 min.
   Note: TrypLE can also be used for cell dissociation.
   Note: Incubation time may vary depending on the matrix.
4) Pipette the cells to fully dissociate and transfer cells to a 15-ml tube filled with 500 µl of Basic03 supplemented with bFGF (Basic03+F) containing Y-27632 (final concentration: 10 µM).
5) Count the cells with a cell counter or hemocytometer (optimized for the cell types).
6) Centrifuge the tubes at 300 *g* at room temperature for 4 min.
7) Aspirate the medium and resuspend cells at a density of 1,000 cells/µl.
8) Aspirate the Geltrex solution and add 1.5 ml of Basic03+F containing Y-27632/well (final concentration: 10 µM).

9) Add 10-20 µl of resuspended cells directly to the new well (10,000-20,000 cells/well).

10) Culture the cells at 37°C in a 5%CO_2 incubator >24 hours.

11) Aspirate the medium and add 1.5 ml of Basic03+F

12) Perform medium changes with 1.5 ml of Basic03+F.

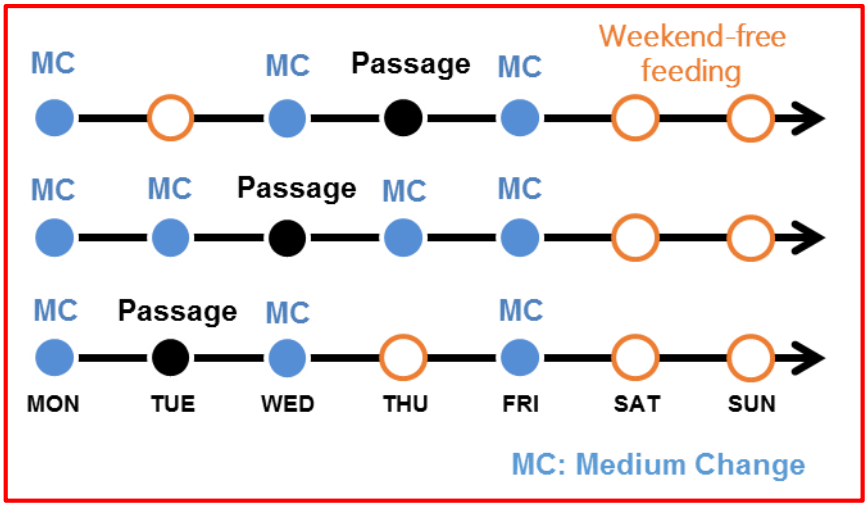
13) Passage the cells every 7 d.

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

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.**

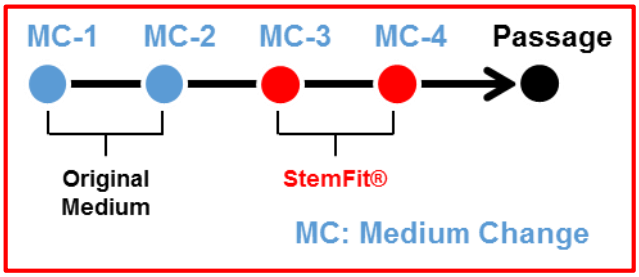
StemFit® Passage Schedule Examples (Weekend-free feeding)



4. Transfer from other culture systems

- To transfer cells from other culture systems to the StemFit® system, we recommend passaging with the original culture system then switching the culture medium to Basic03 supplemented with bFGF (Basic03+F) 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 (~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 37°C