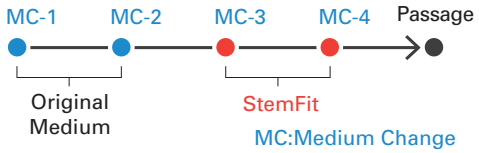
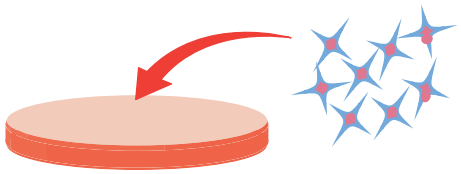
Tips on transitioning cells to StemFit medium

- Switch culture medium to StemFit 2 – 3 days prior to passage

<Example>



- Seed the cells at a higher density (>1.0 x 10^5 cells per well (6-well plate))



For further information, please contact

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Feeder-free medium for ES/iPS cells

StemFit Technical tips

Key points for successful single-cell passage

Benefit 1

Robust and reproducible culture

Quantitative culture

Benefit 2

High fold expansion

～100X expansion / passage

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● Single-cell passage brief protocol example (6-well plate) and tips

StemFit Technical tips

1

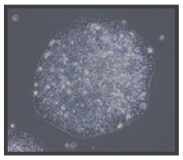
Aspirate the medium and wash once with 2 mL of PBS

2

Add 500 µl/well of Accutase and incubate at 37 °C for 10 min

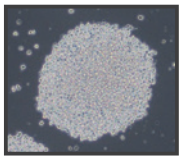
* TrypLE™ can also be used for cell dissociation
* Incubation time may vary depending on the matrix

・**Before incubation with Accutase**



Point-1 **10min!**

・**Gaps in the colonies appear and dissociation of colonies is apparent**

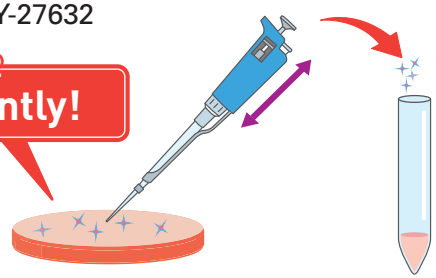


Whole colony can be smoothly detached

3

Gently pipette the cells to fully dissociate and transfer cells to a 15-ml tube filled with 500 µl of culture medium containing 10 µM Y-27632

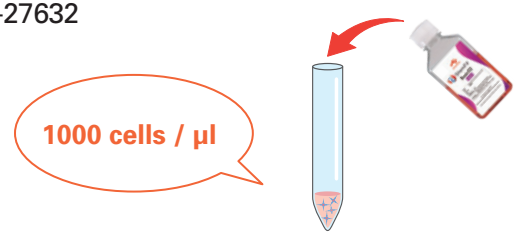
Point-2 **Gently!**



4

Count the cells and centrifuge the tubes

Aspirate the medium and resuspend cells with culture medium containing 10 µM Y-27632



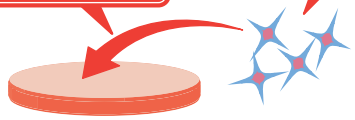
5

Add 10-20 µl (1.0-2.0 x 10^4 cells) of resuspended cells per well in 1.5 mL of culture medium containing 10 µM Y-27632

* It is important to adjust the plating cell number for different lines of hPSCs
* Try a higher seeding density when cell or colony quantity is insufficient (See also Tips on transitioning cells to StemFit medium)

Point-3 **Adjust the Cell Number!**

Point-4 **Distribute evenly!**

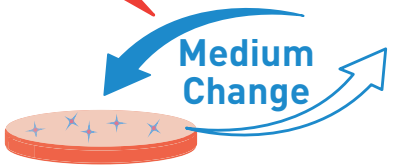


* Immediately distribute the cells evenly over the plate surface to avoid uneven attachment

6

After >24 hours of culture, replace with fresh culture medium without Y-27632

Point-5 **>24 hours!**

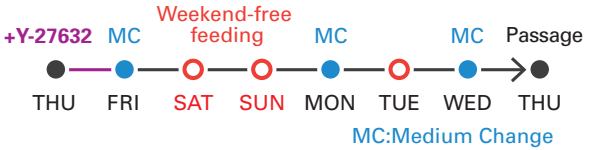


* It is critical that cells are cultured in Y-27632 containing medium for more 24 hours

7

Perform medium change

<Passage Schedule Example>



Point-6
*** If the color of the medium turns orange or yellow, it should be changed every day**

Point-7
*** Do not allow cells to become confluent**

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