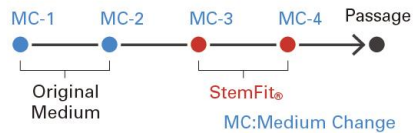


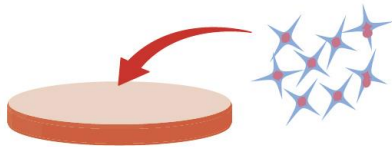
● Tips on transitioning cells to StemFit® medium

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

<Example>



- Seed the cells at a higher density (>1.0 x 10⁵ cells per well (6-well plate))



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

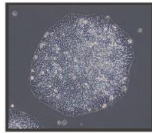
● Single-cell passage brief protocol example (6-well plate) and tips

1 Aspirate the medium and wash once with 2 mL of PBS



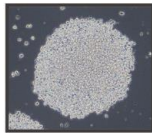
2 Add 500 µl/well of Accutase and incubate at 37 °C for 10 min
 * TrypLE™ can also be used for cell dissociation
 * Incubation time may vary depending on the matrix

• Before incubation with Accutase



Point-1
10 min!

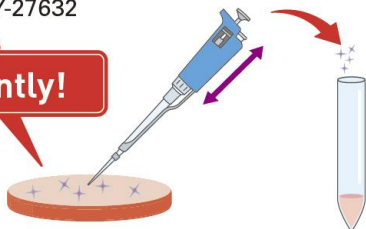
• Gaps in the colonies appear and dissociation of colonies is apparent



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

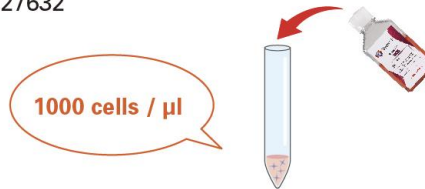
Point-2
Gently!



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 10⁴ 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 higher seeding density when cell or colony quantity is insufficient
 (See also Tips on transitioning cells to StemFit® medium)

Point-4
Distribute evenly!

Point-3
Adjust the Cell Number!



* 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

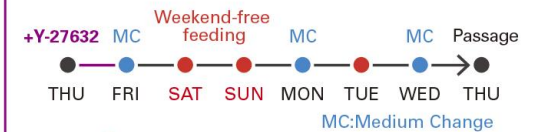
Point-5
>24 hours!



* 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