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

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

Key points for successful single-cell passage

ROBUST AND REPRODUCIBLE CULTURE
Quantitative culture

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 2ml of PBS

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

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

4. Count the cells and centrifuge the tubes
   - 300g RT 4 min
   - Aspirate the medium and resuspend cells with culture medium containing 10 μM Y-27632
   - 1000 cells / μl

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

6. After >24 hours of culture, replace with fresh culture medium without Y-27632
   - It is critical that cells are cultured in Y-27632 containing medium for more than 24 hours

7. Perform medium change

<Passage Schedule Example>

<table>
<thead>
<tr>
<th>MC</th>
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<th>MC</th>
<th>Passage</th>
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<tbody>
<tr>
<td>THU</td>
<td>FRI</td>
<td>SAT</td>
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<td>MON</td>
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</tbody>
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MC: Medium Change

* If the colour of the medium turns orange or yellow it should be changed every day
* Do not allow cells to become confluent

POINTS

POINT 1: 10 min!
POINT 2: Gently!
POINT 3: Adjust the cell number
POINT 4: Distribute evenly!
POINT 5: >24 hours!
POINT 6: * If the colour of the medium turns orange or yellow it should be changed every day
POINT 7: * Do not allow cells to become confluent