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Handling Tips

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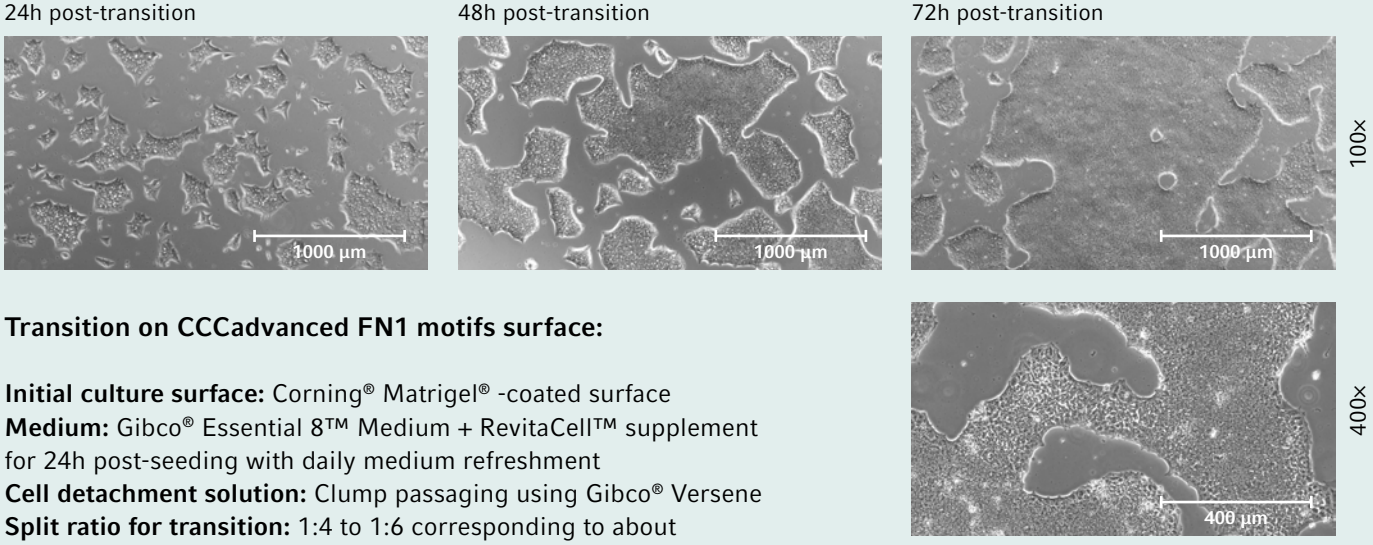
Successful Transition and Expansion of hiPSCs on Eppendorf CCCadvanced™ FN1 Motifs Surface

Cell transition on FN1 motifs surface is quite straightforward. Due to sensitivity of certain pluripotent stem cell lines and the variety of original culture systems consisting of media and growth surface, a surface change may require some adaptation phase.

Tips for successful transition on FN1 motifs:

> Consider 3-5 passages for adaptation (Figure 1).
> Use a stable hiPSC population of high quality at 60-90% confluence for cell seeding.
> Chose a higher initial cell density than usually used during your routine culture (split ratios of 1:4 to 1:6, corresponding to 1 to 1.5 × 10^5 cells per cm^2). Decrease it progressively until complete cell adaptation on FN1 motifs is reached.
> Using a cryopreserved cell stock for transition, thaw cells into the original used culture system and initiate surface transition after full recovery from thawing in the original culture system.
> Prefer clump passaging using Versene®, EDTA, or Gentle solutions for sensitive cell lines. If desired, change your passaging method once the hiPSC culture is stable on FN1 motifs.
> Remove differentiated areas manually before passaging, if spontaneous differentiation occurs.
> Supplement your media with ROCKi for 24h post seeding.
> Avoid changing any other culture conditions (e.g. new medium) during the transition phase. Let cells adapt to the new growth surface before initiating another culture condition change.
> Perform a daily feeding and microscopical check of your hiPSCs.



Transition on CCCadvanced FN1 motifs surface:

Initial culture surface: Corning® Matrigel® -coated surface
Medium: Gibco® Essential 8™ Medium + RevitaCell™ supplement for 24h post-seeding with daily medium refreshment
Cell detachment solution: Clump passaging using Gibco® Versene
Split ratio for transition: 1:4 to 1:6 corresponding to about 1 to 1.5 × 10^5 cells per cm^2

Figure 1: hiPSC morphology following surface transition from Matrigel-coated surface to FN1 motifs surface.

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Tips for successful long-term expansion on FN1 motifs:

> Passage hiPSCs at 70 to 80% of confluency. Initiate passaging earlier if colonies become too dense or if enhanced spontaneous differentiation is observed.

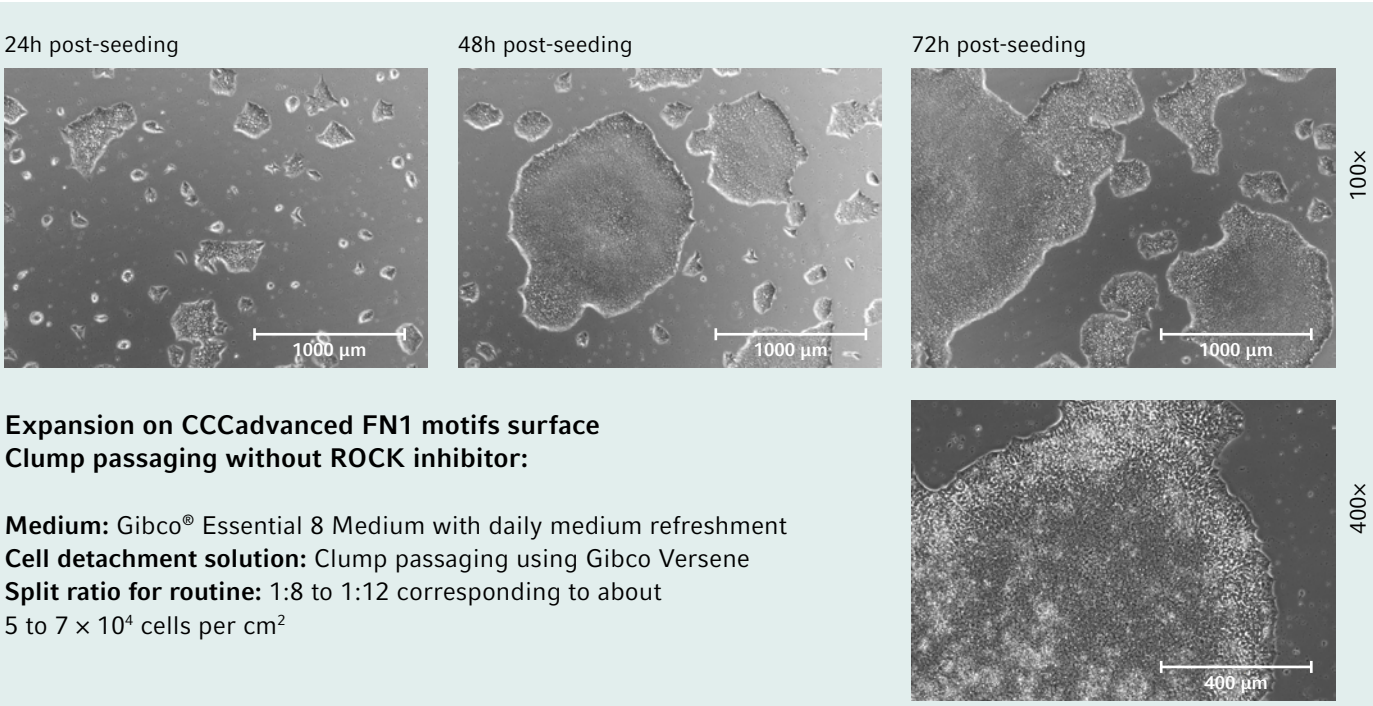
> Remove differentiated areas manually before passaging, if spontaneous differentiation occurs.

> Typical split ratios on FN1 motifs surface are 1:8 and 1:12 (corresponding to 5 to 7 × 10^4 cells per cm^2) performed every 3 to 4 days of culture. Using different split ratios in parallel allow optimal cell population selection for next passage.

> Choose either clump or single-cell passaging methods for routine hiPSC expansion.

> ROCKi is not required for long-term expansion using the clump passaging method (Figure 2).

> Use ROCKi supplementation for 24h post-seeding during single-cell passaging (Figure 3).



Expansion on CCCadvanced FN1 motifs surface
Clump passaging without ROCK inhibitor:

Medium: Gibco® Essential 8 Medium with daily medium refreshment
Cell detachment solution: Clump passaging using Gibco Versene
Split ratio for routine: 1:8 to 1:12 corresponding to about 5 to 7 × 10^4 cells per cm^2

Figure 2: hiPSC morphology post-seeding using clump passaging without ROCK inhibitor supplementation on FN1 motifs surface.

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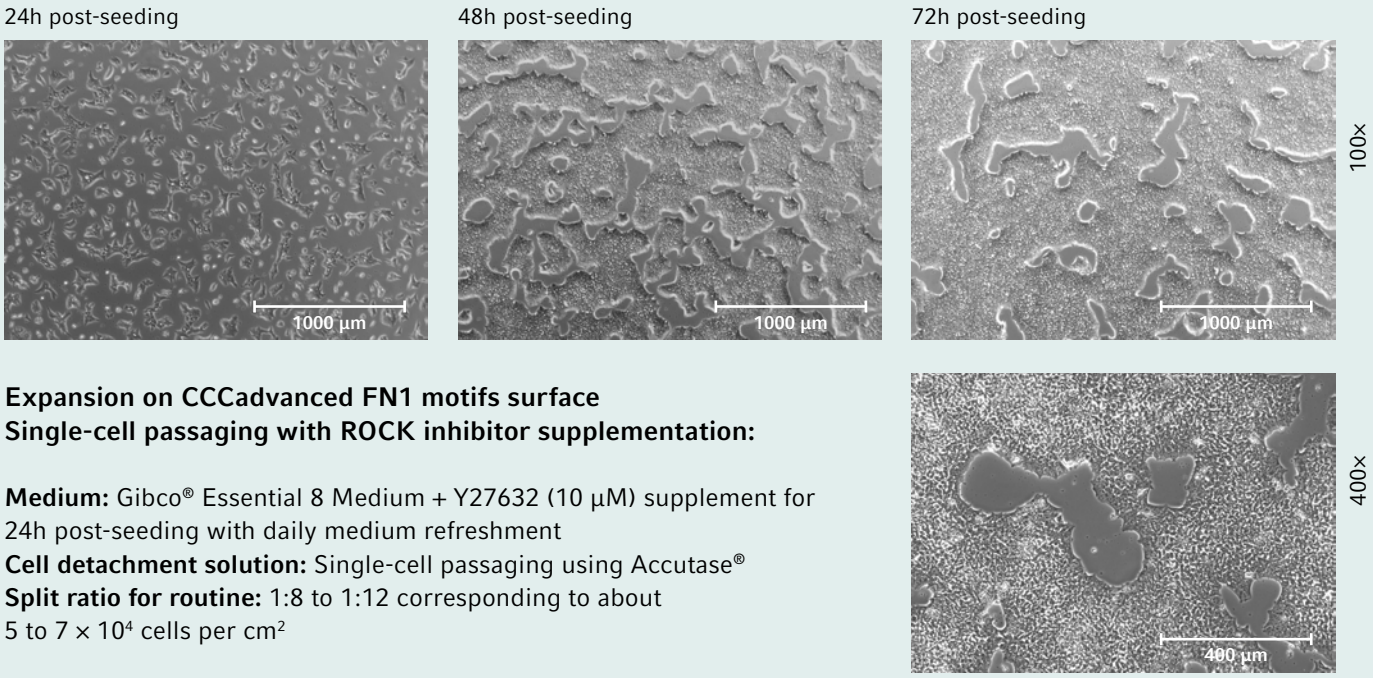

Expansion on CCCadvanced FN1 motifs surface
Single-cell passaging with ROCK inhibitor supplementation:

Medium: Gibco® Essential 8 Medium + Y27632 (10 µM) supplement for 24h post-seeding with daily medium refreshment
Cell detachment solution: Single-cell passaging using Accutase®
Split ratio for routine: 1:8 to 1:12 corresponding to about 5 to 7 × 10^4 cells per cm^2

Figure 3: hiPSC morphology post-seeding using single-cell passaging with ROCK inhibitor supplementation on FN1 motifs surface.

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