

Product No. 21979

AscleStem® hPSC Maintenance Supplement

Product description and precautions before use

- AscleStem® hPSC Maintenance Supplement is a feeder-free culture supplement for maintaining undifferentiated human pluripotent stem cells (hPSCs).
- This product is intended for research use only.
- This product contains components purified from human blood, and have been confirmed to be free from anti-HIV-1 antibodies, anti-HIV-2 antibodies, HBs antigen, anti-HCV antibodies, and anti-HTLV-1 antibodies, and have also tested negative for the presence of HBV, HCV, and HIV by nucleic acid amplification tests. However, the possibility of viruses present below the detection limits testing methods used cannot be completely ruled out. Also, the absence of contamination by other pathogens cannot be completely guaranteed. Therefore, please take necessary protective measures from infection, such as wearing protective equipment, and sterilization with disposal of the product.
- Complete medium is prepared by adding this product to basal DMEM/Ham's F-12 medium, and should be used within 2 weeks. Alternatively, store the supplement as described in the example below. Avoid additional freeze-thaw cycles. This product should be stored frozen (-20°C) and freshly prepared complete medium should be kept refrigerated (4°C), protected from light.
- Since this product prepared under aseptic conditions, sterilization is not required when performing mixing procedures under aseptic conditions.
- When warming the complete medium before use, avoid rapid warming or thawing at 37°C, gently warm it to room temperature.
- Prolonged exposure to room temperature accelerates the deterioration of the medium. After use, promptly store at 4°C.

Preparation of complete medium

1. Gently thaw AscleStem® hPSC Maintenance Supplement at 4°C and mix thoroughly.
2. To prepare fresh complete medium, the entire content of this product (32 mL) to 500 mL of the basal medium (DMEM/Ham's F-12 (code:11581-15)) and mix thoroughly.
3. Store the complete medium at 4°C and use within 2 weeks (*1).
4. Before use, pre-warm the complete medium to room temperature. (Avoid rapid warming using a water bath, etc.)

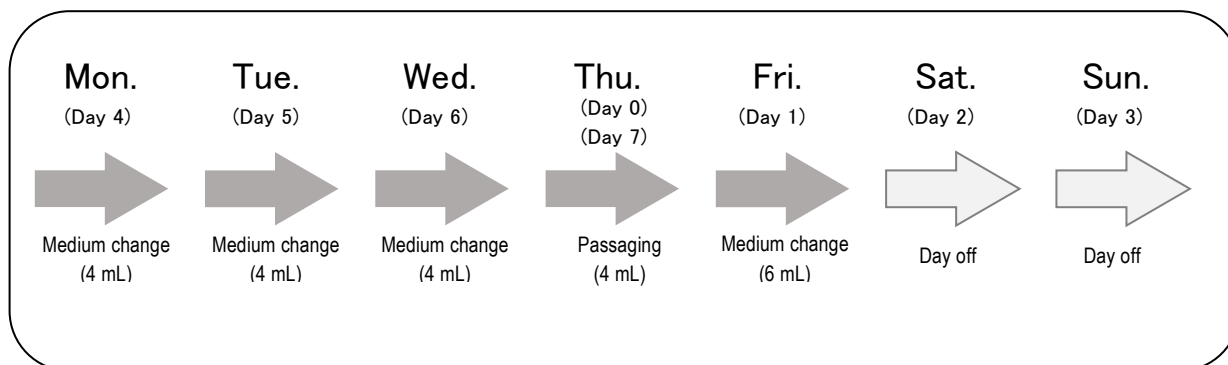
*1 If you cannot use the entire amount of the complete medium within 2 weeks, please divide AscleStem® hPSC Maintenance Supplement into appropriate aliquots following the example below. Avoid freezing and thawing the complete medium as it may cause precipitation.

Example:

1. Divide the thawed product into 2.5 mL aliquots on ice and promptly freeze for storage. Avoid additional freeze-thaw cycles.
2. For complete medium, take 39 mL of basal medium (DMEM/Ham's F-12 (code:11581-15)) in a 50 mL conical tube, add one aliquot (2.5 mL) of the divided product, and mix thoroughly

Culture protocol overview using AscleStem® hPSC maintenance medium

The following overview schedule is an example of a standard weekend-free culture protocol in a 60-mm culture dish.



Required reagents and materials in this culture protocol

- AscleStem® hPSC Maintenance Supplement (code: 21979-44)
- DMEM/Ham's F-12 with L-Gln and Sodium Pyruvate, without HEPES, liquid (code: 11581-15)
- 10 mmol/L Y-27632 solution (code: 18190-54)
- D-PBS(-) without Ca and Mg (code: 14249-95)
- iMatrix-511 (Nippi code: 892011) or Vitronectin (VTN-N) Recombinant Human Protein, Truncated (Thermo Fisher Scientific code: A14700)
- Accumax™ (code: 17087-54) or 0.5 mmol/L EDTA/PBS solution (code: 13567-84)
- AscleStem® Dissociation Solution for hPSCs (code: 21777-84)

Culture protocol for hPSCs using AscleStem® hPSC maintenance medium

Depending on the cell line and experimental endpoint (undifferentiation/Expansion or differentiation), combination(s) of passaging methods (single cell method or clump method) and coating agents (such as Laminin-511 E8 fragments, vitronectin, fibronectin, etc.) may vary and require optimization. This protocol outlines a single cell passaging method using Laminin-511 E8 fragments and clump-passaging method using vitronectin. iPSC 201B7 and 253G1 cell lines can be maintained in an undifferentiated state using either method, but some iPS cell lines, such as Tic cells may require clump-passaging method with vitronectin for proper maintenance of the undifferentiated state. Therefore, if maintenance of undifferentiated cells is unsuccessful, consider use of other passaging methods and coating agents to optimize cell culture/maintenance.

1. Single cell passaging method (253G1, 201B7, etc.)

<Coating culture vessels and preparation before passaging>

- (1) Coat the culture vessels following instructions included with iMatrix-511. Recommended coating concentration is 0.5 $\mu\text{g}/\text{cm}^2$ at 37°C for 1 hour, at room temperature for 3 hours, or at 4°C overnight.
- (2) Prewarm the complete medium to room temperature.
- (3) Remove the coating solution prepared in (1) and add 5 mL of the complete medium and 5 μL of 10 mmol/L Y-27632 solution (final concentration 10 $\mu\text{mol}/\text{L}$, referred to as "new medium" from hereon) to the culture vessels. Place in a 37°C, 5% CO₂ incubator.

<Passaging (Day 0)>

- (1) Remove the plate of cultured human PSCs from the 37°C, 5% CO₂ incubator and aspirate the old medium.
- (2) Wash once with 2 mL of D-PBS (-), add 1 mL of Accumax™ to evenly disperse it across the entire cell surface of the plate, and incubate at 37°C for 3 minutes.
- (3) After 3 minutes, remove the plate from the incubator, tilt to evenly disperse Accumax™ again, and further incubate at 37°C for 2 minutes.
- (4) Following Accumax™ treatment, remove the plates containing the cells as well as the plate containing 5 mL of new medium prepared in the previous step (3) from the incubator.
- (5) Pipette the cells treated with Accumax™ using the leftover Accumax™ liquid in the plate with a 1000 μL pipette to detach the cells.
- (6) Once approximately 80–90% of the adhered cells are detached, add 1 mL of new medium from the previous step (4) and gently pipette while detaching the cells to create a single-cell suspension.
- (7) Perform cell counting procedures and seed the cells at a density of 30,000 to 100,000 cells / 60 mm dish in the plate with new medium. Adjust the seeding cell number appropriately based on the cell line and culture conditions.
- (8) Place the seeded plate into a 37°C, 5% CO₂ incubator to grow and maintain the cells.

<Medium change (Day 1, 4, 5, 6)>

- (1) Aspirate the old medium and add 6 mL of pre-warmed complete medium on Friday (Day 1) or 4 mL on other days to the plate (refer to the above schedule).

2. Clump passaging method (Tic, etc.)

<Coating the culture vessels and preparation before passaging>

- (1) Coat the culture vessels following instructions/manufacture's recommendation included for Vitronectin (VTN-N). Recommended coating concentration is 0.5 $\mu\text{g}/\text{cm}^2$ at room temperature for 1 hour.
- (2) Prepare the required amount of complete medium for passaging in a 50 mL conical tube and add 10 mmol/L Y-27632 solution and prewarm it to room temperature. From now on, this medium will be referred to as "new medium." (For passaging one 60 mm ϕ dish, 12 mL of complete medium and 12 μL of 10 mmol/L Y-27632 solution will be needed. (The final concentration of Y-27632 will be 10 $\mu\text{mol}/\text{L}$))

<Passaging (Day 0)>

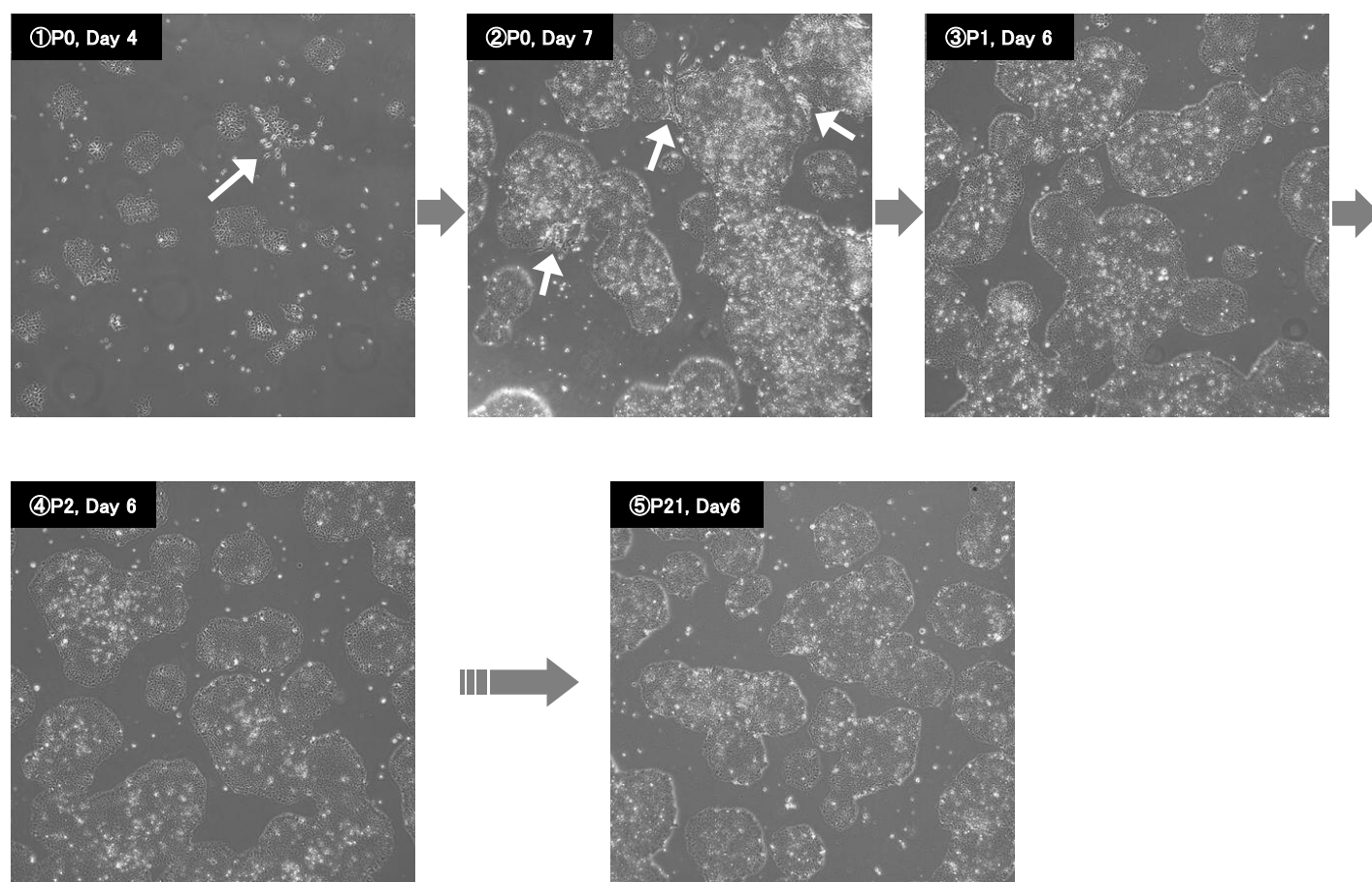
- (1) Remove the plate of cultured human PSCs from the 37°C, 5% CO₂ incubator and aspirate the old medium.
- (2) Wash once with 2 mL of D-PBS (-). Add 2 mL of 0.5 mmol/L EDTA/PBS solution to evenly disperse it across the entire cell surface of the plate and incubate at 37°C for 3 minutes.
- (3) Take the plate out of the incubator, tilt it to evenly disperse the EDTA/PBS solution, and further incubate at 37°C for 4 minutes.

- (4) Remove the plate after EDTA/PBS solution treatment from the 37°C, 5% CO₂ incubator and aspirate the EDTA/PBS solution.
- (5) Add 2 mL of new medium to the plate with adhered cells, pipette (3–4 times) to detach the cells, and collect the cells in a 50 mL conical tube.
- (6) Repeat the process in (5). Then, discard the plate along with the remaining cells that did not detach without applying excessive force.
 - * Adjust the reaction time and pipetting to achieve cell clumps of 50–200 μ m in size. If the clumps are too small, cell viability may decrease, and if they are too large, spontaneous differentiation may occur.
- (7) Centrifuge the tube containing the collected cells at 160 $\times$ g for 3 minutes at room temperature.
- (8) Remove the coating solution from the 6 cm dish coated with Vitronectin (VTN-N) and add 4 mL of new medium.
- (9) After centrifugation, remove the supernatant from the tube, resuspend the cell pellet by tapping.
- (10) Add 4 mL of new medium to the resuspended cell pellet and create a cell suspension.
- (11) Take 10–100 μ L of the cell suspension and seed it into the 6 cm dish prepared in step (8). Return the dish to the 37°C, 5% CO₂ incubator.

<Medium Exchange (Day 1, 4, 5, 6)>

- (1) Aspirate the old medium and add 6 mL of complete medium on Friday (Day 1) or 4 mL on other days to the plate (refer to the above schedule).
 - * Adjust the number of passing times per week (once or twice) depending on the amount of cell suspension seeded in step (11).

<Morphology of human iPS cell line 253G1 cultured on iMatrix-511 in AscleStem® hPSC maintenance medium.>



Switching from another manufacturer's feeder-free undifferentiated hPSC maintenance medium to AscleStem® hPSC maintenance medium may cause changes in the culture environment that can lead to deviation from the undifferentiated state in some colonies after the medium switch (indicated by arrows in photos ① and ②). Such cells tend to become less observable with continued culture and passaging (photos ③, ④, and ⑤). This phenomenon may not occur at all, and it seems to vary between human iPSC cell lines, as well as culture conditions before the medium switch, and the state of the cultured cells. If the switch from another Manufacturer's feeder-free undifferentiated maintenance medium to AscleStem® hPSC maintenance medium is unsuccessful, please consider using the transition method from the on-feeder culture conditions to the product procedures described below.

3. Transition from the on-feeder culture system to the feeder-free culture system with AscleStem® hPSC maintenance medium

(60 mm dish)

- (1) Coat a 60 mm culture dish following the instructions included with iMatrix-511 or Vitronectin (VTN-N).
- (2) Remove the coating solution from the coated 60 mm dish and add 7 mL of complete medium and 7 μ L of 10 mmol/L Y-27632 solution (final concentration 10 μ mol/L). Place it in a 37°C, 5% CO₂ incubator. From now on, this medium will be referred to as "new medium."
- (3) Remove the old medium from the human PSCs cultured in the on-feeder culture system.
- (4) Wash once with 2 mL of D-PBS (-), add 1 mL of AscleStem® Dissociation Solution for hPSCs (code: 21777-84) to evenly disperse it across the entire cell surface of the dish.
- (5) Place it in a 37°C, 5% CO₂ incubator and incubate for 5 minutes. (If the cells do not detach easily in step (7), extend the reaction time.)
- (6) After the reaction, if the edge of the colonies where detachment is observed by microscope, aspirate AscleStem® Dissociation Solution for hPSCs from and wash with 2 mL of D-PBS (-).
- (7) Take 2 mL of new medium prepared in step (2) using a 5 mL pipette and collect the cells by pipetting them into a new 50 mL conical tube.
- (8) Take 1 mL of new medium prepared in step (2) using a 5 mL pipette and resuspend the remaining cells in the dish and collect them in the same 50 mL conical tube from step (7).
- (9) Centrifuge the 50 mL conical tube containing the collected cells at 160 × g for 3 minutes at room temperature and remove the supernatant using an aspirator.
- (10) Gently tap the cell pellet to resuspend.
- (11) Add 1 mL of new medium prepared in step (2) to the cell pellet using a 1000 μ L micropipette, and gently pipette 5–10 times.
- (12) Seed 50–500 μ L (depending on the cell type, cell number, and culture schedule of the iPS cell line) of the cell suspension obtained in step (11) into the 60 mm dish prepared in step (2).
- (13) Place it in a 37°C, 5% CO₂ incubator and continue culturing. Then, follow the above passaging schedule and perform medium change and passaging procedures accordingly.

Storage

Storage temperature can be found on the product label.

Expiration

Expiration date can be found on the product label.

Reference

Ying Hua, Kenji Yoshimochi, Junjun Li, Kazuhiro Takekita, Motoshi Shimotsuma, Lingjun Li, Xiang Qu, Jingbo Zhang, Yoshiki Sawa, Li Liu, Shigeru Miyagawa
Development and evaluation of a novel xeno-free culture medium for human-induced pluripotent stem cells
Stem Cell Research & Therapy, volume 13, Article number: 223 (2022)

AscleStem® hPSC Maintenance Supplement is a collaborative product from open innovation project between Osaka University and Nacalai Tesque, Inc.

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