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Cellular Dynamics international

Essential 6™ Medium

Description

Essential 6™ Medium is a fully-defined, xeno-free medium, which supports reprogramming of somatic cells and the differentiation of human pluripotent stem cells. Essential 6™ Medium requires the addition of basic fibroblast growth factor (bFGF) when reprogramming human cells.

Product	Catalog No.	Amount	Storage	Shelf Life*
Essential 6™ Medium	A1516501	500 mL	Store at 2–8°C. Protect from light	12 months

* Shelf Life duration is determined from Date of Manufacture.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture Conditions

Media: Essential 6™ Medium

Cell Line: Human pluripotent stem cells (PSCs)

Temperature Range: 37°C

Incubator Atmosphere Range: Humidified atmosphere of 5% CO_2

Culture Type: Adherent

Recommended Culture Vessels: Induced pluripotent stem cells (iPSCs) can be derived and/or differentiated in complete Essential 6™ Medium on vitronectin (VTN-N)-coated, tissue culture-treated vessels.

Ensure proper gas exchange and minimize exposure of cultures to light.

Derivation of Induced Pluripotent Stem Cells (iPSCs) in Essential 6™ Medium

Reprogramming Fibroblasts using Episomal iPSC Reprogramming Vectors

Day –4 to –2: Plate human fibroblasts into a T75 flask in fibroblast medium so that they are 75–90% confluent on the day of transfection (Day 0).

Day 0: Transfect the cells using the Neon® Transfection System. Plate transfected cells onto vitronectin-coated culture dishes and incubate overnight in Essential 8™ Medium supplemented with hydrocortisone (1 µM).

Day 1 to Day 5–10: Replace the spent medium with fresh Essential 8™ Medium with hydrocortisone (1 µM); change the spent medium every other day.

Day 5–10 to Day 25–30: Replace the medium with Essential 6™ Medium supplemented with bFGF (100 ng/mL). Continue culturing the cells, changing the spent medium every other day.

Day 25–30: Pick, transfer, and change the medium to Essential 8™ Medium.

Reprogramming Fibroblasts using CytoTune™-iPS Sendai Reprogramming Kit

Day –2: Two days before transduction, plate human neonatal foreskin fibroblast cells into two wells of a 6-well plate at the appropriate density to achieve 80–90% confluency per well on the day of transduction (Day 0).

Day 0: Perform transduction.

Day 1: 24 hours after transduction, replace the medium with fresh fibroblast medium. Culture the cells for 5 more days, changing the spent medium with fresh fibroblast medium every other day.

Day 6: Replace the medium with Essential 6™ Medium supplemented with bFGF (100 ng/mL).

Day 7: Harvest cells and seed on vitronectin-coated (1 µg/cm^2) plates using Essential 6™ Medium supplemented with bFGF (100 ng/mL); replace the spent medium every day thereafter.

Day 8 to 28: Feed and monitor the cells. When colonies are ready for transfer, perform live staining using Tra1-60 or Tra1-81 to select reprogrammed colonies. Manually pick colonies and transfer them onto prepared vitronectin-coated plates and culture them in Essential 8™ Medium.

Note: Colonies are typically ready to be picked at Day 21, but they may require a few additional days depending on the somatic cell line.

Identifying iPSC colonies

By Day 21 post-transduction, the cell colonies on the vitronectin-coated plates are large and compact, covering the majority of the surface area of the culture vessel. However, only a fraction of these colonies will consist of iPSCs, which exhibit a hESC-like morphology characterized by a flatter cobblestone-like appearance with individual cells clearly demarcated from each other in the colonies. Therefore, we recommend that you perform live staining with Tra1-60 or Tra1-81 antibodies that recognize undifferentiated iPSCs.

Picking iPSC colonies

1. Place the culture dish containing the reprogrammed cells under an inverted microscope and examine the colonies under 10X magnification.
2. Mark the colony to be picked on the bottom of the culture dish.

   Note: We recommend picking at least 10 distinct colonies by the end of each reprogramming experiment and expanding them in separate 24-well culture plates.
3. Transfer the culture dish to a sterile cell culture hood (i.e., biosafety cabinet) equipped with a stereomicroscope.
4. Using a 25-gauge 1½-inch needle, cut the colony to be picked into 5–6 pieces in a grid-like pattern.
5. Using a 200 µL pipette, transfer the cut pieces to one well of a freshly prepared 24-well vitronectin coated culture plate containing human Essential 8™ Medium.
6. Incubate the culture plate containing the picked colonies in a 37°C incubator with a humidified atmosphere of 5% CO_2.
7. Allow the colonies to attach to the culture plate for 48 hours before replacing the spent medium. After that, change the medium every day.
8. Treat the reprogrammed colonies like normal human ESC colonies and passage, expand, and maintain them using standard culture procedures until you have frozen cells from two 60-mm plates.

Embryoid Body (EB) formation using Essential 6™ Medium

Note: We recommend picking at least 10 distinct colonies by the end of each reprogramming experiment and expanding them in separate 24-well culture plates.

1. Observe the human iPSCs growing in Essential 8™ Medium under the microscope to confirm that the cells are 70–80% confluent and ready to be subcultured.
2. Cut out and remove any differentiated colonies prior to passaging the culture.
3. Pre-warm the required volume of Dispase® solution (2 mg/mL) and Essential 6™ Medium in a 37°C water bath for 15 minutes.
4. Aspirate the spent medium from the culture dish using a pipette, and rinse the cells twice with DPBS, no calcium, no magnesium.
5. Gently add pre-warmed Dispase® solution to the culture dish (e.g., 1 mL of Dispase® solution per 60-mm culture dish). Swirl the culture dish to coat the entire cell surface.
6. Incubate the culture dish at 37°C for 3 minutes.
7. Remove the dish from the incubator, aspirate the Dispase® solution, and gently wash the cells with DPBS, no calcium, no magnesium.
8. Gently scrape the cells off the surface of the culture dish using a cell scraper, and transfer the cells to a sterile 15-mL centrifuge tube.
9. Rinse the culture dish twice with DPBS, no calcium, no magnesium, gently "spraying off" any cells that have not detached. Pool the rinse with the cells in the 15-mL tube.
10. Centrifuge the tube at 200 × *g* for 5 minutes at room temperature to pellet the cells.
11. Carefully aspirate the supernatant without disturbing the cell pellet and discard it.
12. Gently flick the tube to fully dislodge the cell pellet from the tube bottom, and gently resuspend the cells in pre-warmed Essential 6™ Medium using a 5-mL serological pipette. Do not triturate.

    Note: It is critical to gently resuspend the cells without using force to avoid damage.
13. Transfer the cells onto a 60-mm or a 100-mm non-tissue culture-treated dish (i.e., the EB dish).
14. Place the EB dish in a 37°C incubator with a humidified atmosphere of 5% CO_2 in air.
15. Change the medium on the EBs every other day by transferring the entire volume of the dish into a centrifuge tube. Keep the tube in the hood and allow the cells to settle to the bottom of the tube (about 5 minutes). Then, using a pipette, remove the supernatant from the tube and replace it with fresh Essential 6™ Medium. Place the cells back onto the same dish.
16. Continue to change the medium every other day. The EBs will grow in size over time.
17. After 7 days, transfer the cells into a 100-mm Geltrex®-coated tissue culture-treated dish to allow for attachment. Incubate the EBs in a 37°C, 5% CO_2 incubator.
18. Replace spent medium every other day.
19. Allow the cells to expand for 14–21 days, or even longer. The entire EB dish can be immunostained or harvested for analysis by PCR at 14 days, 21 days, or later.

For detailed protocols, visit
www.lifetechnologies.com/protocols.

Related Products

Product	Cat. No.
Essential 8™ Medium	See below*
Vitronectin, truncated human recombinant (VTN-N)	A14701
Episomal Reprogramming Vectors	A14703
CytoTune™-iPS Sendai Reprogramming Kit	A13780
Geltrex™ LDEV-Free hESC-qualified Reduced Growth Factor Basement Membrane Matrix	A14133
DPBS, no calcium, no magnesium	14190
Dispase®, powder	17105
FGF-Basic (AA 1-155) Recombinant Human Protein	PHG0261

* Refer to www.lifetechnologies.com/Essential8.

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

				
Caution, consult accompanying documents	Temperature Limitation	Protect from light	Use By:	Consult instructions for use
				
Batch Code	Catalog number	Manufacturer	Sterilized using aseptic processing techniques	

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support.
For further assistance, email techsupport@lifetech.com

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