ExCellerate™ iPSC Expansion Medium

Animal Component-Free Medium for Human Pluripotent Stem Cell Culture

Catalog Number: CCM036

Volume: 500 mL

PRODUCT DESCRIPTION

ExCellerate iPSC Expansion Medium is a complete, specifically formulated and optimized medium for *in vitro* feeder-free expansion of undifferentiated human pluripotent stem cells, including embryonic and induced pluripotent stem cells. No materials of animal or human origin are used in the production of this medium or its components. This medium is compatible with a variety of cell culture matrices including Cultrex™ Ultimatrix (R&D Systems®, Catalog # BME001), ReadyBME (R&D Systems, Catalog # 3434-050-RTU) and Recombinant Human Vitronectin (R&D Systems, Catalog # 2308-VN).

STABILITY & STORAGE

Upon receipt, store the medium at ≤ -20 °C until use or the expiration date on the Certificate of Analysis. Thaw the medium overnight at 2-8 °C. **Never thaw the medium at 37 °C.** Upon thawing, the medium may be stored at 2-8 °C for two weeks. For longer storage, aliquot and store the medium at ≤ -20 °C until the expiration date on the CofA. Do not expose the medium to repeated freeze-thaw cycles.

PRECAUTIONS

When handling bio-hazardous materials, safe laboratory procedures should always be followed, and PPE should be worn. Acute and chronic effects of over-exposure to this medium are unknown.

The protocols below describe the expansion of human pluripotent stem cells using ExCellerate iPSC Expansion Medium, in combination with either Cultrex Basement Membrane Extract (BME) or Recombinant Human Vitronectin. This protocol is optimized for 6 cm culture dishes but can be scaled according to tissue culture surface area.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This reagent should not be used beyond the expiration date indicated on the Certificate of Analysis.
- Results may vary due to variations among pluripotent stem cell populations derived from different donors.

REGULAR FEEDING OF iPSCs

1. Warm only the necessary volume of ExCellerate iPSC Expansion Medium at room temperature for 20-30 minutes.
   Note: ***Never warm media at 37 °C.***
2. Aspirate the spent media from the culture dish, leave a little bit so that the dish does not dry out.
3. Gently add 2-2.5 mL of fresh media to each 6 cm culture dish.
4. Change the media daily and monitor cell health. Passage before cells reach 80% confluency.

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THAWING iPSCs INTO EXCELLERATE™ iPSC EXPANSION MEDIUM

1. Prepare a 6 cm dish coated with either Cultrex™ Ultimatrix, ReadyBME, or Vitronectin.
2. Warm only the required volume of ExCellerate iPSC Expansion Medium at room temperature for 20-30 minutes. Add 5-10 µM of Y-27632 (Tocris™, Catalog #1254) to the medium.
3. Quickly thaw the frozen cells in a 37 °C water bath and transfer the cells to a 15 mL tube.
4. Slowly add 6 mL of fresh ExCellerate iPSC Expansion Medium with Y-27632 to the cells to prevent osmotic shock.
5. Centrifuge cells at 200 x g for 5 minutes.
6. Aspirate the supernatant and add 2 mL of ExCellerate iPSC Expansion Medium containing Y-27632, then transfer cells to the coated culture dish. Move the dish in a cross configuration (south to north, then east to west) a few times to distribute the cells evenly across the dish. Swirling the dish in a circle can create an uneven distribution of cells along the outside of the dish.
7. Check the health of the cells the next day and replace medium with fresh iPSC medium without Y-27632.
8. Change the medium daily and monitor cell health.

CLUMP PASSAGING OF iPSCs

For regular subculturing, we recommend performing clump passaging of pluripotent stem cells.

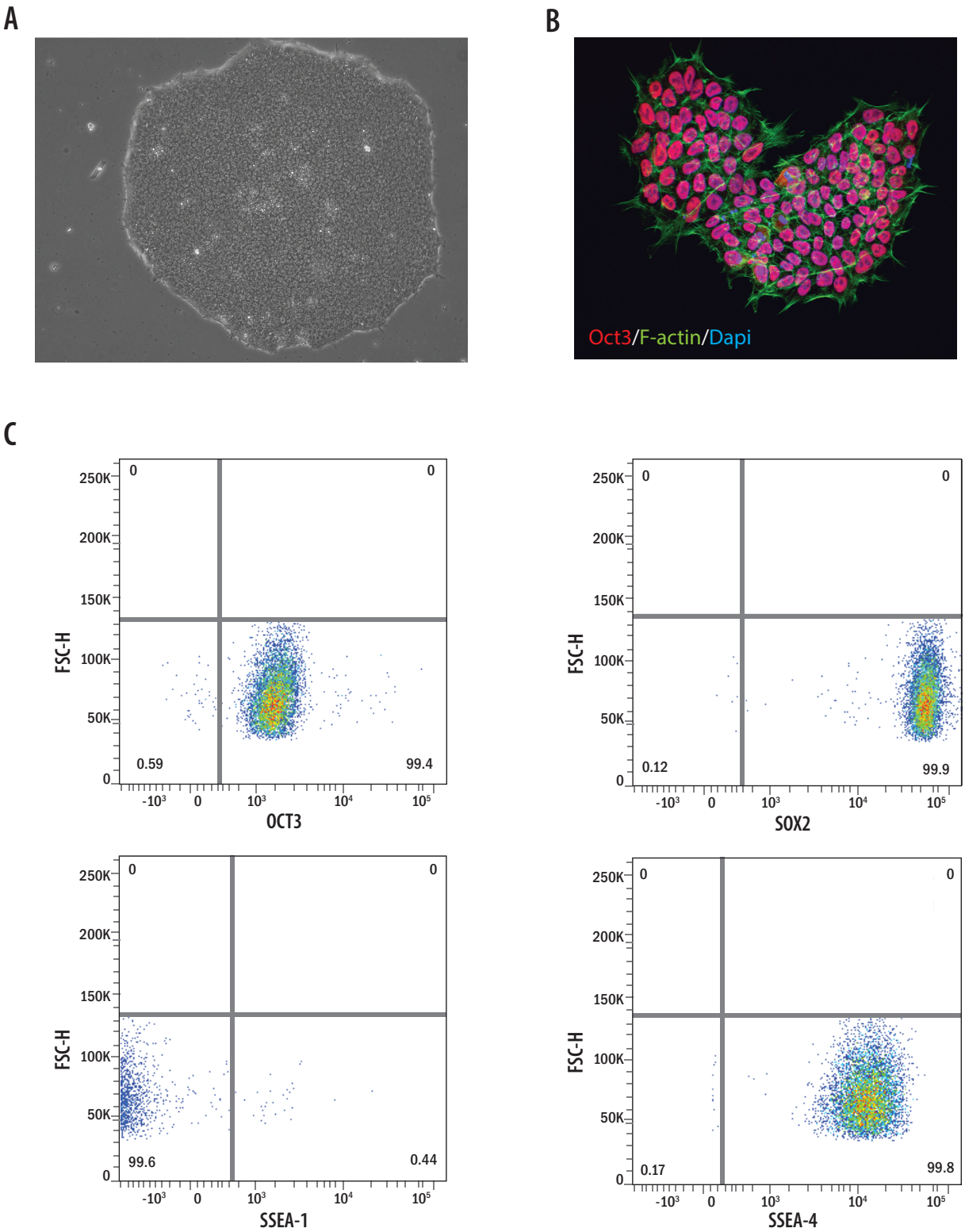
1. Prepare a 6 cm dish coated with either Cultrex Ultimatrix, ReadyBME, or Vitronectin.
2. Warm ExCellerate iPSC Expansion Medium and an EDTA solution (such as Versene) at room temperature for 20-30 minutes.
3. Aspirate cell media, wash once with 1 mL of EDTA solution or D-PBS and then add 1 mL of EDTA solution and incubate at room temperature for 6-10 minutes.
4. Remove the EDTA solution and add 1 mL of iPSC expansion media.
   Note: *Most cells should still be lightly adherent to the dish and only lift off once medium is added. It is not uncommon to lose a little bit of cells with clump passaging. If many colonies appear to be floating from the surface, pipette the EDTA solution up and down to dislodge the rest of the cells. Collect the cells in a 15 mL conical tube. Centrifuge cells at 200 x g for 5 minutes. Aspirate the supernatant and add 1 mL of medium to the cells and then proceed to Step 6.*
5. Gently pipette cells up and down (5-8 times to fully dislodge the cells).
6. Aspirate coating solution from dishes and then add cells at desired split ratio.
7. Move the dish in a cross configuration (south to north, then east to west) a few times to distribute the cells evenly across the dish.
8. Change the media daily and monitor cell health. Passage the cells at the desired confluency.

SINGLE CELL PASSAGING OF iPSCs

For applications such as differentiation where having an accurate cell count is critical, we recommend performing single cell passaging.

1. Prepare a 6 cm dish coated with either Cultrex Ultimatrix, ReadyBME, or Vitronectin.
2. Warm the ExCellerate iPSC Expansion Medium (add 5-10 µM of Y-27632) at room temperature for 20-30 minutes.
3. Aspirate media from cultured cells and add 1 mL of Accutase® and incubate at 37 °C or room temperature for 5-10 minutes.
4. Gently pipette cells up and down to dislodge clumps. Transfer suspension into a 15 mL conical tube.
5. Rinse plate with ~3-5 mL of ExCellerate iPSC Expansion Medium containing Y-27632. Add suspension to conical with cells.
6. Centrifuge cell suspension at 200 x g for 5 minutes.
7. Remove the supernatant and add 1 mL of iPSC medium containing Y-27632. Count cells using a hemocytometer.
8. Aspirate coating solution, add 2 mL of medium containing Y-27632 to the dish. Plate cells at desired concentration. Move the dish in a cross configuration (south to north, then east to west) a few times to distribute the cells evenly across the dish.
9. Check the health of the cells the next day and replace medium with fresh iPSC medium without Y-27632.

DATA EXAMPLES



Examples of iPSC colony maintenance in ExCellerate™ iPSC Expansion Medium. hiPSC lines cultured in ExCellerate iPSC Expansion Medium show healthy rounded colony morphology, compact cells and clearly defined smooth colony edges **(A)** and express high levels of the stemness marker OCT3/4 assessed by immunohistochemistry staining of OCT3/4 (red), F-actin (green) and DAPI (blue) **(B)** hiPSCs also express high levels of undifferentiated stem cell makers OCT3/4, SOX2, SSEA-4 and low levels of differentiated stem cell marker SSEA-1 assessed by flow cytometry (R&D Systems®, Catalog # FMC001) **(C)**.