ExCellerate™ iPSC Expansion Medium Animal-Free, GMP

Catalog Number: CCM036-GMP

Volume: 500 mL

PRODUCT DESCRIPTION

ExCellerate iPSC Expansion Medium, Animal Free, GMP is a complete, specifically formulated medium for *in vitro* feeder-free expansion of undifferentiated human pluripotent stem cells, including embryonic and induced pluripotent stem cells. This media was produced in a GMP animal free facility with no materials of animal or human origin used in the production of this medium or its components.

STABILITY & STORAGE

Upon receipt, store the medium at ≤ -20 °C until 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.

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.

LIMITATIONS

- For pre-clinical or *ex vivo* clinical use.
- 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.

OTHER MATERIALS REQUIRED

- Cell Adhesion Substrate such as Cultrex™ UltiMatrix (Catalog # BME001), ReadyBME (Catalog# 3434-050-RTU), or Vitronectin
- Pipettes and pipette tips
- 6 cm culture dishes
- 15 mL conical tubes
- 37 °C, 5% CO_2 incubator
- Inverted microscope
- Cell Counter
- Centrifuge
- Cell harvesting solution (e.g. EDTA based such as Versene™ or Accutase®)
- D-PBS
- ROCK Inhibitor (Tocris™ Y-27632, Catalog # TB1254-GMP)
  Alternatively; CEPT Cocktail available from Tocris, (Catalog # 7163), Emricasan (Catalog # 7310), Polyamine Supplement x1000 (lyophilized) (Catalog # 7739), and Trans-ISRIB (Catalog # 5284)

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PROCEDURE FOR THE EX VIVO CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS

The protocols below describe the expansion of human pluripotent stem cells using ExCellerate™ iPSC Expansion Medium, Animal Free, GMP in combination with either Cultrex™ Basement Membrane Extract (BME) or Recombinant Human Vitronectin. This protocol is optimized for 6 cm culture dish using the ROCK Inhibitor Y-27632 (Tocris™, Catalog #1254) but can be adjusted to use other culture vessels and ROCK inhibitors.

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 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.

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 mL of fresh media to each dish.
4. Change the media daily and monitor cell health. Passage before cells reach 80% confluency.

THAWING iPSCs INTO EXCELLERATE™ iPSC EXPANSION MEDIUM

1. Prepare coated 6 cm dishes.
2. Warm only the required volume of ExCellerate iPSC Expansion Medium with Y-27632 at room temperature for 20-30 minutes.
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 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 iPSC expansion medium with Y-27632, then transfer cells to the coated culture dish.
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. Check the health of the cells the next day and replace medium with fresh iPSC medium without Y-27632.
9. 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 coated 6 cm dishes.
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 approximately 5 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 in the EDTA solution, then pipette the EDTA solution up and down to dislodge the rest of the cells. Collect the cells in a 15 mL conical tube. Pellet the cells by centrifuging at 200 x g for 5 minutes. Aspirate the supernatant and add 1 mL of iPSC expansion 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 previously prepared dishes, add 2 mL of fresh iPSC expansion medium to the 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 coated 6 cm dish.
2. Warm the ExCellerate iPSC Expansion with 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, until cells have dislodged as single cells. Gently pipette cells up and if necessary.
4. Transfer suspension into a 15 mL conical tube. Rinse plate with approximately 3-5 mL of ExCellerate iPSC Expansion Medium with Y-27632 to collect as many cells as possible. Add suspension to conical with cells.
5. Centrifuge cell suspension at 200 x g for 5 minutes.
6. Remove supernatant, add 1 mL of iPSC expansion medium with Y-27632 and count cells.
7. Aspirate coating solution, add 2 mL of iPSC expansion medium with Y-27632 to the dish. Plate cells at desired concentration.
8. 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 daily with fresh iPSC medium without Y-27632.

END USER TERMS OF USE OF PRODUCT

Production records and facilities are available for examination by appropriate personnel on site at R&D System in Minneapolis, Minnesota USA.

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