Human iPS Growth Medium (PSGro Medium, TBS8023)

Product Overview

Tribio™ Human iPS Growth Medium (**PSGro Medium**) is designed for human induced pluripotent stem cells (iPSCs) and human embryonic stem cells (ESCs). It is serum-free and chemically-defined as all of its ingredients are synthetic chemicals or purified recombinant proteins. PSGro Medium supports feeder-free growth and maintenance of human iPSCs or human ESCs on extra-cellular matrix proteins (e.g., BD Matrigel™). Its performance is comparable to the market-leading mTeSR™1 in growth rate, characteristic morphology, pluoripotent markers, normal karyotype, and differentiation to germ layers.

Content and Storage

PSGro (Cat. # TBS-8023) contains two components:

Components	Size	Storage	Shelf Life
Basal Medium (on gel ice)	450 mL	2 to 8°C. Keep from light	6 months
10x Supplement (dry ice)	50 mL	-20°C or below. Keep from light	6 months

Additional key reagents required or suggested

- ●Extracellular matrix proteins, such as Matrigel™ (BD, cat# 354277) or equivalent
- ●Accutase (Millipore, SCR005) or Collagenase IV (Invitrogen, 17104) or equivalent
- ●ROCK inhibitor Y-27632 (StemRD, Y-01/-05/-25) or Thiazovivin (StemRD, Thia-01/-05/-25)

Medium Preparation

1. Thaw PSGro Supplement at room temperature or overnight at 4°C. If desired, thawed Supplement can be Aliquoted and stored at -20°C or below, but further freeze-thaw should be avoided.
2. To make PSGro complete medium, add 1 part of Supplement to 9 parts of Basal Medium. Mix.
3. Antibiotics can be added to the complete medium if so desired.
4. Once made, PSGro complete medium is stable for 2 weeks when stored in dark at 2 to 8°C.

Coating plates with extracellular matrix proteins, such as Matrigel™

Refer to manufacturers' instructions. For BD Matrigel™, the following procedure can be followed:

1. Thaw Matrigel™ on ice. Dilute Matrigel 1:50 with pre-chilled DMEM medium.
2. Immediately use the diluted Matrigel™ solution to coat tissue culture plates. For a 6-well plate, use 1 mL of diluted Matrigel™ solution per well, and swirl the plate to spread the solution evenly.
3. Let the plate stand for 1 h at 37°C or overnight at 4°C.

Adaptation of growing feeder-free cells to PSGro

Human iPSC or ESCs that have been grown in most commonly-used hiPSC/ESC media can be easily adapted into PSGro. One round of 1:1 dilution of serum-containing or serum-free medium with PSGro is generally sufficient. However, for certain media and cell lines, a longer and more graduate adaptation may be required.

Adaptation of growing feeder-dependent cells to PSGro

1. Dissociate cells with Accutase, Collagenase IV or equivalent.
2. Add the previously-used medium and gently scrape off cells as aggregates. *The ideal aggregate size is similar to other media and single cells from excessive pipetting may lower plating efficiency*.
3. Transfer the suspension with cell aggregates into a 15 ml tube.
4. Centrifuge at 200 x g for 5 minutes at room temperature.
5. Discard Matrigel™ solution, immediately add PSGro (e.g., 2 mL/well for a 6-well plate).
6. After centrifugation, discard the supernatant, gently resuspend the pellet with 2 mL of the previously-used medium. ***Note: Adding ROCK inhibitor (Y-27632 or Thiazovivin) to the medium is shown to increase the survival and plating efficiency.***
7. Transfer the cell aggregates in the previously-used medium to the wells with PSGro. Mix. Gently.
8. Culture the cells at 37°C, with 5% CO2 and 95% humidity.
9. On the next day, change medium to 4 mL fresh PSGro

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Recovery of frozen cells in PSGro

1. Quickly thaw hiPSCs or hESCs in a 37°C water bath, transfer the contents to a 15 mL tube. Add 10 mL of the medium previously-used for the cells drop-wise to the tube with gentle mixing.
2. Centrifuge cells at 200 *x g* for 5 minutes at room temperature.
3. Discard Matrigel™ solution, immediately add PSGro (e.g., 2 mL/well for a 6-well plate).
4. After centrifugation, discard the supernatant, gently resuspend the pellet with 2 mL of the previously-used medium. ***Note: Adding ROCK inhibitor (Y-27632 or Thiazovivin) to the medium is shown to increase the survival and plating efficiency.***
5. Transfer the cell aggregates in the previously-used medium to the plate with PSGro. Mix gently.
6. Culture the cells at 37°C, with 5% CO2 and 95% humidity.
7. The next day, change medium to 4 mL fresh PSGro.

Passaging cells in PSGro under feeder-free condition

1. Look under microscope to identify regions of differentiation. Mark the differentiated colonies using lens marker on the bottom of the plate (e.g., 6-well plate)
2. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Aspirate medium from the cell culture and rinse with PBS.
4. Add 0.5 mL per well of Accutase or Collagenase IV. Let stand at room temp for 1 – 2 min.
5. Remove Accutase, and gently rinse 2 – 3 times with 2 mL PSGro to remove remaining enzymes.
6. Add 2 mL/well PSGro and scrape colonies off with a cell scraper.
7. Transfer the detached cell aggregates to a 15 mL conical tube and rinse the well with an additional 2 mL PSGro to collect any remaining aggregates.
8. Centrifuge at 200 x *g* for 5 minutes at room temperature.
9. Aspirate the supernatant. Resuspend pellet in 4 mL PSGro by pipetting gently. ***Note: Adding ROCK inhibitor (Y-27632 or Thiazovivin) to the medium is shown to increase the survival and plating efficiency.***
10. Plate the cell aggregates with PSGro onto a new plate coated with Matrigel™.
11. Culture the cells at 37°C, with 5% CO2 and 95% humidity.
12. Change medium daily. *Note: If the colonies are at an optimal density, the cells can be split every 5 - 7 days using 1:5 to 1:10 splits.*

Cryopreservation of cells in PSGro

1. Prepare freezing medium which contains 90% PSGro and 10% DMSO. ***Note: Adding ROCK inhibitor (Y-27632 or Thiazovivin) to the medium is shown to increase the survival and plating efficiency.***
2. Perform steps 1 - 8 from **Passaging cells in PSGro under feeder-free condition**
3. Gently aspirate the supernatant taking care to keep the cell pellet intact.
4. Gently resuspend the pellet in freezing medium, taking care to leave the cell aggregates larger than would normally be done for passaging.
5. Transfer 1 mL of cell aggregates in freezing medium into each labeled cryovial.
6. Place vials into an isopropanol freezing container and place the container at -80°C overnight.
7. Transfer to a liquid nitrogen tank the next day for long-term storage.

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