Tribo™ Brown Adipocyte Differentiation Kit (TBS8028)

Product Overview

Tribo™ Brown Adipocyte Differentiation Kit is designed for brown adipocyte derivation from human pluoripotent stem cells (ESC or iPSC) grown as a monolayer culture. The kit contains all necessary serum-free media formulated with polypeptide differentiation factors and modulators of the key adipogenic pathways in a 3-step procedure. It is optimized to use with the serum-free and feeder-free growth medium **PSGro Medium** (TBS8023).

Package Size, Content and Storage

Kit has two package sizes

- **Starter** kit: TBS8028-10: 3 wells (6-well plate), ~$5x10^6$ starting cells
- **Regular** kit: TBS8028- 50: 15 wells (6-well plate), ~$2.5x10^7$ starting cells

Components (5)	*Intended Outcome*	***Starter** Kit Size*	***Regular** Kit Size*
PSGro Plus Medium	ESC or iPSC preparation	30 mL	130 mL
Part A	mesoderm derivation	10 mL	50 mL
Part B	Hematopoietic precursor derivation	10 mL	50 mL
Part C	Brown adipocyte progenitor derivation	10 mL	50 mL
BAGro Medium	BA maturation & maintenance	55 mL	250 mL

Storage: 2 to 8°C. Keep from light. Do NOT freeze. **Shelf Life:** 1 month if stored as directed.

Other reagents required:

1. **Matrigel™**: for cell plating
2. **Accutase** or equivalent: for cell splitting
3. Phosphate Buffered Saline (**PBS, TBS5003**): for cell washing
4. ROCK inhibitor **Thiazovivin** (StemRD # Thia) or Y-27632 (# Y27632): for optimal cell plating
5. **PSGro Medium**(TBS8023) or equivalent (e.g., mTeSR®): for hESC/iPSC maintenance
6. **Oil Red O Solution** (Electron Microscopy Sciences, cat# 26609): for oil droplet staining
7. PCR reagents for brown adipocyte markers (e.g., UCP-1 and PRDM16)

Cell Preparation in PSGro Plus Medium

Coating plates with Matrigel™: Refer to manufacturer's instruction.
Recovery of frozen cells in PSGro Medium: Refer to PSGro Medium User Manual (TBS8023) for details.

Adaptation of growing cells to PSGro Medium

Most human ESC or iPSC lines that have been cultured as feeder-dependent or feeder-independent culture can be adapted to PSGro Medium. Refer to PSGro Medium User Manual (TBS8023) for details.

Cell plating in PSGro Plus Medium

1. Start from a routine culture of hESC or hiPSC in **PSGro Medium** or a similar serum-free medium (e.g., mTeSR®). Identify and remove differentiated cells by scraping and aspiration.
2. Aspirate the medium and rinse twice with PBS.
3. Add 0.5 mL of Accutase per well (6-well plate). Incubate at 37°C for 3 – 5 min and verify that colonies have become single cells or small clusters (2 – 10 cells/cluster) under microscope.
4. Add 2 mL/well **PSGro Plus Medium** and pipet up & down 2 – 3 times gently.
5. Transfer the detached cells to a conical tube. Centrifuge at 200 x g for 5 minutes at room temp.
6. Aspirate the supernatant. Resuspend pellet in 3 mL **PSGro Plus Medium** gently. Note: adding **Thiazovivin** (2.5 uM) or **Y-27632** (10 uM) to **PSGro Plus Medium** at this step markedly increases plating efficiency.
7. Plate the cells in a Matrigel™-coated well. For most cell lines, a 1:5 to 1:10 splitting from a routine, confluent, culture may be appropriate while the ideal split may vary between lines.
8. Culture at 37°C, 5% CO_2 / 95% humidity. Refresh with **PSGro Plus Medium** (without ROCKi) daily.

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Adiopocyte Differentiation

Cell density at the onset of differentiation: cell density is critical to achieve optimal brown adipocyte differentiation. The ideal cell density at the onset of induction is **40 - 60%** confluency. Less confluent culture may suffer from excessive cell loss upon induction whereas more confluent culture may result in over-confluency at the end of the procedure. Usually, if cells are plated in **PSGro Plus Medium** as recommended, they should reach 40 - 60% confluency in 2-3 days.

A. Warm **Part A** to room temp. Aspirate PSGro Plus Medium, rinse once with PBS. Add 3 mL of **Part A**, incubate the cells at 37°C, 5% CO_2/95% humidity for **2** day.

Expected result: As a result of differentiation, cells become bigger and flatter.

B. Warm **Part B** to room temp. Aspirate **Part A**. Add 3 mL **Part B**, incubate the cells for **2** days. Do not change the medium.

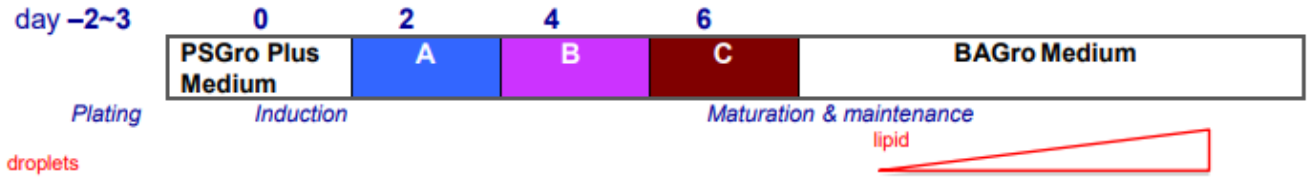
Expected result: Cell morphology continues to change. Some cell dislodging may occur, but the overall confluency increases as cells are becoming bigger and flatter.

C. Warm **Part C** to room temp. Aspirate **Part B**. Add 3 mL **Part C**, incubate the cells for **2** days. Do not change the medium.

Expected result: Cells become oval or rounded in shape and some cell dislodging may continue.

D. Warm **BAGro Medium** to room temp. Aspirate **Part C**. Add 2 mL **BAGro Medium**, and incubate the cells for 2 – 3 days. Refresh the medium every 2 – 3 days.

Expected result: Cells shape stabilizes as rounded or oval. Lipid droplets typically appear after 4 – 8 day in **BAGro** Medium (day 8 ~ 12 from induction) and increase over the next few days. Maturation and survival of the resulting brown adipocytes are expected over the next several weeks.



Trouble-shooting

1. Excessive amount of cell dislodging or over-confluence: This usually occurs when cell density is too low or too high at the onset of induction. Start the induction at 40% to 60% confluence.

2. Low efficiency: A large number of factors influence adiopogenic efficiency of a particular pluoripotent stem cell line. The main determinants include:
   - The quality of the starting cells: it is crucial to maintain cells at their full pluripotent state before induction.
   - The well-known diversity between pluoripoent cell lines, especially iPSC lines. Since ESC lines are generally more amenable to differentiation, a strongly-adiopogenic ESC line should be included as a control.

3. If no lipid droplet or brown adipocyte-specific gene expression appears after **2 weeks**, the procedure has likely failed. As adiopogenic differentiation is governed by a large number of factors, many of which are out of operators' control, success cannot be guaranteed for all cell lines under every setting.

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