

Tribo™ Brown Adipocyte Differentiation Kit (TBS8028)

Catalog	Unit Size
TBS8028-10	Starter Kit
TBS8028-50	Regular Kit

Description

Tribo™ Brown Adipocyte Differentiation Kit is designed for brown adipocyte derivation from human pluripotent 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 with the serum-free and feeder-free growth medium **PSGro Medium** (TBS8023).

Kit Size has two package sizes

Starter kit: TBS8028-10: 3 wells (6-well plate), $\sim 5 \times 10^6$ starting cells

Regular kit: TBS8028- 50: 15 wells (6-well plate), $\sim 2.5 \times 10^7$ starting cells

Components (5)	Intended Outcome	Starter Kit	Regular Kit
PSGro Plus Medium	ECS or iPSC Preparation	30 mL	130 mL
BA Part A	Mesoderm derivation	10 mL	50 mL
BA Part B	Hematopoietic Precursor derivation	10 mL	50 mL
BA Part C	Brown adipocyte progenitor derivation	10 mL	50 mL
BAGro Medium	BA Maturation and maintenance	55 mL	250 mL

Storage

Store at -20°C for 1 year.

Other reagents required

1. **Matrigel™** for cell plating
2. **Accutase (TBS8089)** or equivalent: for cell splitting
3. Phosphate Buffered Saline (**PBS, TBS5003**): for cell washing
4. ROCK inhibitor **Thiazovivin** or Y-27632 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

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

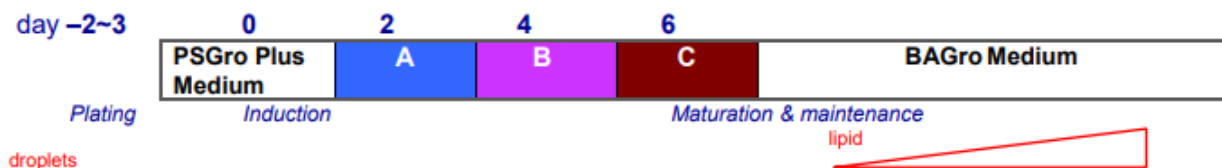
1. Start from a routine culture of hESC or hiPSC in **PSGro Medium** or a similar serum-free medium (e.g., mTeSR®). Identify and remove differentiated cells by scraping and aspiration.
2. Aspirate the medium and rinse twice with PBS.
3. Add 0.5 mL of Accutase per well (6-well plate). Incubate at 37°C 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 $200 \times g$ for 5 minutes at room temperature.
6. Aspirate the supernatant. Resuspend pellet in 3 mL **PSGro Plus Medium** gently. Note: adding **Thiazovivin** (2.5 μM) or **Y-27632** (10 μM) 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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Adipocyte 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 **BA Differentiation A** to room temperature. Aspirate PSGro Plus Medium, rinse once with PBS. Add 3 mL of **BA Differentiation A** incubate the cells at 37°C, 5% CO₂/95% humidity for 2 days.
Expected result: As a result of differentiation, cells become bigger and flatter.
- B. Warm **BA Differentiation B** to room temperature. Aspirate **BA Differentiation A**. Add 3 mL **BA Differentiation 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 **BA Differentiation C** to room temperature. Aspirate **BA Differentiation B**. Add 3 mL **BA Differentiation 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 temperature. Aspirate **BA Differentiation 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 round or oval. Lipid droplets typically appear after 4 – 8 days 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.*



Troubleshooting

1. Excessive amount of cell dislodging or over-confluency: This usually occurs when cell density is too low or too high at the onset of induction. Start the induction at 40% to 60% confluency.
2. Low efficiency: A large number of factors influence adipogenic efficiency of a particular pluripotent 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 pluripotent cell lines, especially iPSC lines. Since ESC lines are generally more amenable to differentiation, a strongly-adipogenic 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 adipogenic 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.

Related Product

MCF-7 Cell Complete Medium (TBS8036)
 MCF-10 Cell Complete Medium (TBS8024)
 DMEM Medium (TBS8061)
 RPMI-1640 (TBS8063)
 0.1% Gelatin Solution (TBS8004)
 2x HBS, pH7.05 (TBS5076)
 PSGro Medium (TBS8023).
 Accutase (TBS8089)

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