StemRD
EMPOWERING STEM CELL R&D

332 Beach Road Burlingame, CA 94010 USA
www.stemrd.com PHONE: (650)-343-1888
info@stemrd.com FAX: (650)-343-1889

PSdif-BA® Brown Adipocyte Differentiation Kit

Human brown adipocytes can now be derived from pluoripotent stem cells (ES or iPS cells) by activating the classic brown adiogenic pathways (Ref 1, 2). ***PSdif-BA®*** *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®*** *(StemRD cat# PGro).* *Ref 1: Tseng, et al., Nature 454: 1000, 2008; Ref 2: Nishio, et al., Cell Metab, 16: 394, 2012*

Package Size, Content and Storage

PSdif-BA® has two package sizes:

- **Starter** kit: catalog # PB-STR (or PB-000 for samples): 3 wells (6-well plate), ~5x10^6 starting cells
- **Regular** kit: catalog # PB-REG: 15 wells (6-well plate), ~2.5x10^7 starting cells

Components (5)	*Intended Outcome*	***Starter** Kit Size*	***Regular** Kit Size*
PSGro® Plus	ESC or iPSC preparation	30 mL	130 mL
PSdif-BA® A	mesoderm derivation	10 mL	50 mL
PSdif-BA® B	Hematopoietic precursor derivation	10 mL	50 mL
PSdif-BA® C	Brown adipocyte progenitor derivation	10 mL	50 mL
BAGro®	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**): for cell washing
4. ROCK inhibitor **Thiazovivin** (StemRD # Thia) or Y-27632 (# Y27632): for optimal cell plating
5. **PSGro®** (StemRD # PGro) 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

Coating plates with Matrigel™: *Refer to manufacturer's instruction.*

Recovery of frozen cells in PSGro®: *Refer to PSGro® User Manual on StemRD website for details. https://www.stemrd.com/index.php?/Media/PSGro-Medium/flypage.tpl.html*

Adaptation of growing cells to PSGro®

Most human ESC or iPSC lines that have been cultured as feeder-dependent or feeder-independent culture can be adapted to PSGro®. Refer to PSGro® User Manual on StemRD website for details.

Cell plating in PSGro® Plus

1. Start from a routine culture of hESC or hiPSC in **PSGro®** 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** 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** gently. *Note: adding* ***Thiazovivin*** *(2.5 uM) or* ***Y-27632*** *(10 uM) to* ***PSGro® Plus*** *at this step markedly increases plating efficiency.*

332 Beach Road Burlingame, CA 94010 USA
www.stemrd.com PHONE: (650)-343-1888
info@stemrd.com FAX: (650)-343-1889

7. Plate the cells in a Matrigel™-coated well. For most cell lines, a 1:5 to 1:10 splitting from a routine, sub-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** (without ROCKi) daily.

Differentiation with PSdif-BA®

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** as recommended, they should reach 40 - 60% confluency in 2-3 days.*

A. Warm **PSdif-BA® A** to room temp. Aspirate PSGro® Plus, rinse once with PBS. Add 3 mL of **PSdif-BA® 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 **PSdif-BA® B** to room temp. Aspirate **PSdif-BA® A**. Add 3 mL **PSdif-BA® B**, incubate the cells for **2** days. Do not change the medium.

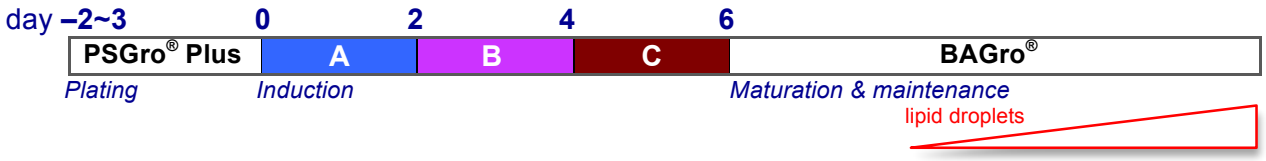
Expected result: Cell morphology continues to change. Some cell dislodging may occur, but the overall confluency increases as cells are becoming bigger and flatter.

C. Warm **PSdif-BA® C** to room temp. Aspirate **PSdif-BA® B**. Add 3 mL **PSdif-BA® 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®** to room temp. Aspirate **PSdif-BA® C**. Add 2 mL **BAGro®**, 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®** (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 StemRD's or operators' control, success cannot be guaranteed for all cell lines under every setting.

For Research Use Only