

# PrimaPure™



A division of Gene Therapy Systems, Inc.

## Human Preadipocytes (HPAd)

Catalog #	Description/Content	Amount
PH80205A	HPAd, Heart, Adult	>500,000 cells
PH80305A	HPAd, Subcutaneous, Adult	>500,000 cells
PH80205AK	HPAd (Heart, Adult) Complete System	1 Kit*
PH80305AK	HPAd (Subcutaneous, Adult) Complete System	1 Kit*

\*Each kit contains an ampoule of cryopreserved HPAd (PH80205A or PH80305A), 500 ml of Human Preadipocyte Growth Medium (PM811500), and a Subculture Reagent Kit (PR090100K).

Related Products	Catalog #
Human Preadipocytes Growth Medium, 500 ml	PM811500
HEPES Buffered Saline Solution (HBSS), 100 ml	PR062100
Trypsin/EDTA, 100 ml	PR070100
Trypsin Neutralizing Solution, 100 ml	PR080100
Subculture Reagent Kit, including 100 ml each of HBSS, Trypsin/EDTA, and Trypsin Neutralizing Solution	PR090100K
GenePORTER 2 Transfection Reagent, 0.75 ml	T202007
GeneSilencer siRNA Transfection Reagent, 200 reactions	T500750
BioPOTER® Protein Delivery Reagent, 24 Reactions	BP502401

<b>Storage:</b>	Store cryopreserved vials in liquid nitrogen immediately upon arrival. Store the growth medium at 4°C in the dark immediately upon arrival. Store the Subculture Reagent Kit at -20°C upon arrival and store the reagents at 4°C upon thawing.
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## INTRODUCTION

Human Preadipocytes (HPAd) are derived from human adipose tissue. These fibroblast-like precursor cells are cryopreserved at the end of primary culture and can be propagated two passages prior to differentiating into Human Adipocytes (HAd). HPAd derived HAd were used to study insulin-stimulated glucose transport<sup>1,2</sup>, growth hormone enhanced lipolysis<sup>3</sup> and obese gene expression<sup>4</sup>. HPAd can be fully differentiated into mature HAd in Adipocyte Differentiation Medium. Significant differences in lipolysis and leptin production<sup>4</sup> are observed in HPAd and HAd from human omental and subcutaneous adipose tissue. We provide HPAd from subcutaneous adipose tissue at various sites and adipose depot on heart. HPAd/HAd are useful in vitro cellular model for investigating the causes and treatment of obesity, type II diabetes and cardiovascular diseases. Recently it was discovered that human adipocytes might be a new target for the immunodeficiency virus-1<sup>5</sup>.

## MATERIALS AND METHODS

### I. Preparation for Culturing

1. Make sure your Class II Biological Safety Cabinet, with HEPA filtered laminar airflow, is in proper working condition.
2. Clean the Biological Safety Cabinet with 70% alcohol to ensure it is sterile.
3. Turn the Biological Safety Cabinet blower on for 10 min. before cell culture work.
4. Make sure all serological pipettes, pipette tips, and reagent solutions are sterile.
5. Follow the standard sterilization technique and safety rules:
  - a. Do not pipette with mouth.
  - b. Always wear gloves and safety glasses when working with human cells even though all the strains have been tested negative for HIV, Hepatitis B and Hepatitis C.
  - c. Handle all cell culture work in a sterile hood.

### II. Culturing HPAd

1. Remove the cryopreserved vial of HPAd from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 1 minute.

4. Take the vial out of the water bath and wipe dry.
5. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
6. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.

### III. Plating Preadipocytes

- A. PREPARING CELL CULTURE FLASKS FOR CULTURING HSkMC
1. Take the Preadipocyte Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
  2. Pipette 15 ml of Preadipocyte Growth Medium\* into a T-75 flask.

\* Keep the medium to surface area ratio at 1 ml per 5 cm<sup>2</sup>. For example, 5-7.5 ml for a T-25 flask or a 60 mm tissue culture dish; 15-20 ml for a T-75 flask or a 100 mm tissue culture dish.

B. THAWING AND PLATING HPAd

1. Remove the cryopreserved vial of HPAd from the liquid nitrogen storage tank using proper protection for your eyes and hands.
2. Turn the vial cap a quarter turn to release any liquid nitrogen that may be trapped in the threads, then re-tighten the cap.
3. Thaw the cells quickly by placing the lower half of the vial in a 37°C water bath for 1 minute.
4. Take the vial out of the water bath and wipe dry.

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5. Decontaminate the vial exterior with 70% alcohol in a sterile Biological Safety Cabinet.
6. Remove the vial cap carefully. Do not touch the rim of the cap or the vial.
7. Resuspend the cells in the vial by gently pipetting the cells 5 times with a 2 ml pipette. Be careful not to pipette too vigorously as to cause foaming.
8. Pipette the cell suspension (1ml) from the vial into the T-75 flask containing 15 ml of Preadipocyte Growth Medium.
9. Cap the flask and rock gently to evenly distribute the cells.
10. Place the T-75 flask in a 37°C, 5% CO<sub>2</sub> humidified incubator. Loosen the cap to allow gas exchange. For best results, do not disturb the culture for 24 hours after inoculation.
11. Change to fresh Preadipocyte Growth Medium after 24 hours or overnight to remove all traces of DMSO.
12. Change Preadipocyte Growth Medium every other day until the cells reach 60% confluent.
13. Double the Preadipocyte Growth Medium volume when the culture is >60% confluent or for weekend feedings.
14. Subculture the cells when the HPAd culture reaches 85-95% confluent.

### C. SEEDING HPAd FOR ASSAY IMMEDIATELY

1. Transfer thawed preadipocyte suspension aseptically to a conical tube containing 15 ml of Preadipocyte Growth Medium.
2. Rinse the cryovial with Growth Medium and add the rinse to the same conical tube.
3. Centrifuge the cells at 220x g for 5 minutes at room temperature.
4. Aspirate the supernatant from the tube without disturbing the cell pellet.
5. Resuspend washed cells in a 2 ml of Preadipocyte Growth Medium by gently pipetting the cells to break up the clumps.
6. Count the cells with a hemocytometer or cell counter.
7. Adjust the volume of Preadipocyte Growth Medium to bring the cell/ml to the desired cell density according to the following chart:

Culture Format	Surface Area/Well	Cell Density cell/ml	Volume per well	# Cells per well	Total Volume
6 well plate	9.40 cm <sup>2</sup>	90,000	4 ml	360,000	24 ml
12 well plate	3.83 cm <sup>2</sup>	75,000	2 ml	150,000	24 ml
24 well plate	1.88 cm <sup>2</sup>	75,000	1 ml	75,000	24 ml
48 well plate	0.86 cm <sup>2</sup>	70,000	500 µl	35,000	24 ml
96 well plate	0.32 cm <sup>2</sup>	90,000	150 µl	13,500	14.4 ml
T-25 flask	25 cm <sup>2</sup>	200,000	5 ml	1,000,000	5 ml
T-75 flask	75 cm <sup>2</sup>	200,000	15 ml	3,000,000	15 ml

8. Seed the preadipocytes at 40,000 cells/cm<sup>2</sup> in the desired format for differentiation.
9. Place the cells in a 37°C, 5% CO<sub>2</sub> humidified incubator.

10. Follow the instructions for differentiating HPAd into HAd in Section V.

## IV. Subculturing HPAd

### A. PREPARING SUBCULTURE REAGENTS

1. Remove the Subculture Reagent Kit from the -20°C freezer and thaw overnight in a refrigerator.
2. Make sure all the subculture reagents are thawed. Swirl each bottle gently several times to form homogeneous solutions.
3. Store all the subculture reagents at 4°C for future use. The activity of Trypsin/EDTA Solution will be stable for 2 weeks when stored at 4°C.
4. Aliquot Trypsin/EDTA solution and store the unused portion at -20°C if only portion of the Trypsin/EDTA is needed.

### B. PREPARING CULTURE FLASK

1. Take the Preadipocyte Growth Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
2. Pipette 35ml of Preadipocyte Growth Medium to a T-175 flask (to be used in Section IV C Step 14.)

### C. SUBCULTURING HPAd

#### Trypsinize Cells at Room Temperature. Do Not Warm Any Reagents to 37°C.

1. Remove the medium from culture flasks by aspiration.
2. Wash the monolayer of cells with HBSS and remove the solution by aspiration.
3. Pipette 6 ml of Trypsin/EDTA Solution into the T-75 flask. Rock the flask gently to ensure the solution covers all the cells.
4. Remove 5 ml of the solution immediately.
5. Re-cap the flask tightly and monitor the trypsinization progress at room temperature under an inverted microscope. It usually takes about 2 to 4 minutes to loosen the cells. The cells may not become completely round during the trypsinization and some cells may maintain some processes even though they are loosened from the culture surface.
6. Release the rounded cells from the culture surface by hitting the side of the flask against your palm until most of the cells are detached.
7. Pipette 5 ml of Trypsin Neutralizing Solution to the flask to inhibit further tryptic activity.
8. Transfer the cell suspension from the flask to a 50 ml sterile conical tube.
9. Rinse the flask with an additional 5 ml of Trypsin Neutralizing Solution and transfer the solution into the same conical tube.
10. Examine the T-75 flask under a microscope. If there are >20% cells left in the flask, repeat Steps 2-9.
11. Centrifuge the conical tube at 220 x g for 5 minutes to pellet the cells.
12. Aspirate the supernatant from the tube without disturbing the cell pellet.
13. Resuspend the cells in 5 ml of Preadipocyte Growth Medium by gently pipetting the cells to break up the clumps.

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- Count the cells with a hemocytometer or cell counter. Inoculate at 15,000 cells per cm<sup>2</sup> for rapid growth, or at 10,000 cells per cm<sup>2</sup> for regular subculturing.
- For setting up assay, follow instructions in Section IV A.

\*HPAd can only be subcultured 2 passages prior to differentiation.

### V. Differentiating HPAd

#### Do Not Let Cell Dry During The Medium Changes.

##### A. PREPARING DIFFERENTIATION MEDIUM

- Take the Adipocyte Differentiation Medium from the refrigerator. Decontaminate the bottle with 70% alcohol in a sterile hood.
- Warm the Adipocyte Differentiation Medium to room

temperature.

##### B. DIFFERENTIATING HPAd TO HAd

- Remove growth medium from culture flask by aspiration.
- Add the appropriate volume of Adipocyte Differentiation Medium according to the chart in Section III C, Step 7.
- Incubate cells in a 37°C, 5% CO<sub>2</sub> humidified incubator in the Adipocyte Differentiation Medium.
- Change to fresh Adipocyte Differentiation Medium every 3 days for 15 days.
- At the end of 15 days, cells are differentiated into HAd with lipid droplets in the cells.
- Remove Adipocyte Differentiation Medium and starve the cells in Adipocyte Starving Medium for 1 day prior to assay.

### REFERENCES

- Tanti, J-F. et al., Endocrinology 138(5):2005 (1997)
- Okada, T. et al., J. Biochem Mol Biol 269(5):3568 (1994)
- Nam, S.Y. et al., Obes Rev 1(2):73 (2000)
- Hamilton, B.S. et al, Nature Medicine 1(9):953 (1995)
- van Harlemen, V. et al., Diabetes 51:2029 (2002)
- Hazan, U. et al., FASEB J. 16(10):1254 (2002).

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