

Poietics™ human ADSCs – adipogenesis protocol

Procedure notes

This procedure is a recommendation only. The ADSC are not quality control tested for differentiation and differentiation is not guaranteed under the cell warranty.

1. For adipose derived stem cell (ADSC) differentiation into adipocytes, cells can either be plated for assays of differentiation directly out of cryopreservation or after passaging.
2. If first subculturing cells, follow the adipose derived stem cell instructions for use. It is recommended to thaw and culture cells in the ADSC basal medium supplemented with the ADSC growth SingleQuots™ kit. When ready to plate cells for adipogenic differentiation follow the section 'Adipogenic assay procedure' below.
3. If plating cells for differentiation directly out of cryopreservation, the preadipocyte basal medium-2 (PBM-2) supplemented with FBS, L-glutamine, and GA-1000 from the PGM™-2 SingleQuots™ kit can be used to thaw and plate cells.

Preparation of media

Preadipocyte growth medium-2 (PGM™-2)

1. Decontaminate the external surfaces of the PGM™-2 SingleQuots™ vials and the preadipocyte basal medium-2 (PBM-2) bottle with 70% v/v ethanol or isopropanol.
2. Aseptically open the bottle of fetal bovine serum (FBS). Add the contents to the 500 ml PBM-2.
3. Aseptically open the L-glutamine and GA-1000 cryovials and add the entire amount from each to the PBM-2.
4. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses should not affect the cell characteristics.

Preadipocyte differentiation medium-2 (PDM-2)

1. Transfer 100 ml of the PGM™-2 prepared above to a new, sterile nalgene medium bottle.
2. Aseptically open the following cryovials and add to the 100 ml of PGM™-2:

- a. rhInsulin
- b. Dexamethasone
- c. IBMX (3-isobutyl-1-methyl-xanthine)
- d. Indomethacin

3. Note that this makes a **2x concentration** of PDM-2 and must be diluted 1:2 (i.e. 0.1 ml PGM™-2 + 0.1 ml PDM-2) for cell culture.

Thawing of cells

1. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten. Quickly thaw the cryovial in a 37°C water bath.
2. Watch your cryovial closely; when the last sliver of ice melts remove it. Do not submerge it completely. Thawing the cells for longer than 1½ minutes results in less than optimal results.
3. Remove the cryovial immediately, wipe it dry, and transfer to a sterile field where the equilibrated flasks should be waiting, ready to seed. Rinse the cryovial with 70% alcohol, and then wipe to remove excess.
4. Using a micropipette, gently add the thawed cell suspension to 5 ml of temperature-equilibrated medium.
5. Centrifuge at 500 x g for 5 minutes at room temperature.
6. Resuspend the pellet in a minimum volume of temperature equilibrated preadipocyte growth medium-2 (PGM™-2) by gently pipetting up and down. Count the total number of viable cells.

Adipogenic assay procedure

Plating cells for adipogenesis

1. For assays of adipogenic differentiation, the recommended seeding density for human adipose derived stem cells is 31,250-62,500 cells per cm² (10,000-20,000 cells/well in a 96-well plate) in 0.625 ml PGM™-2 per cm² (0.2 ml in a 96-well plate).

2. Prepare a cell suspension in PGM™-2 using the recommended seeding density and volume of media per cm² above.
3. Plate cells in the desired multi-well plate.
4. Incubate at 37°C, 5% CO₂ and 90% humidity.
5. When cells have reached ≥90% confluence (typically 24-48 hours after plating), induce differentiation following the instructions below.

Induction of adipogenic differentiation

Adipogenic differentiation medium (PDM-2) should be used once the ADSC have become ≥ 90% confluent. Prepare the medium before ADSC become confluent.

1. Carefully remove all PGM™-2 media from each well.
2. To cells that will remain as undifferentiated controls add fresh PGM™-2 media at 0.625 ml per cm² (i.e. 0.2 ml in a well of a 96 well plate).
3. To cells that will be differentiated add fresh PGM™-2 media at 0.313 ml per cm² then add PDM-2 media at 0.313 ml per cm² to dilute the 2x media to a 1x final concentration.
4. Do not let the cells dry out when changing medium.
5. Incubate at 37°C, 5% CO₂ and 90% humidity for 10-12 days.
6. Media changes are not required during the 10-12 day differentiation period.
7. The extent of adipogenic differentiation may be noted by microscopic observation of lipid vacuoles in the induced cells (see figure 1). To document the adipogenic differentiation, cultures may be assayed using AdipoRed™ assay reagent (see figure 2). Non-induced cells will have few, if any, lipid vacuoles (see figure 3).

Figure 1. ADSC stained with AdipoRed™

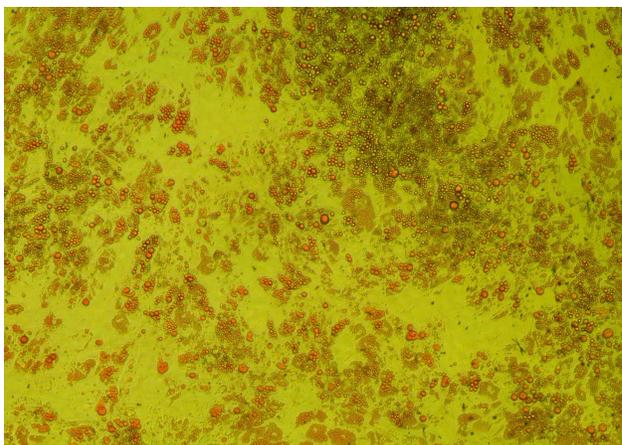


Figure 2. Quantitative analysis of ADSC lipid accumulation via AdipoRed™ assay

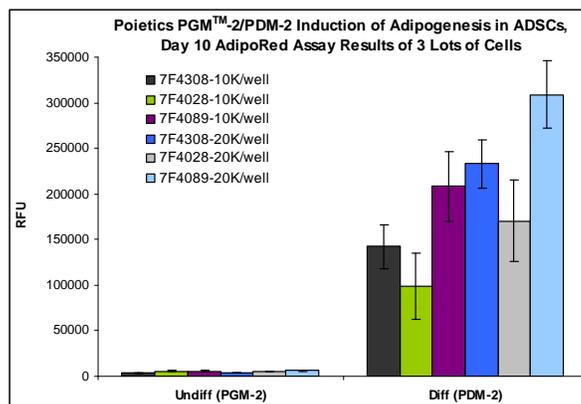
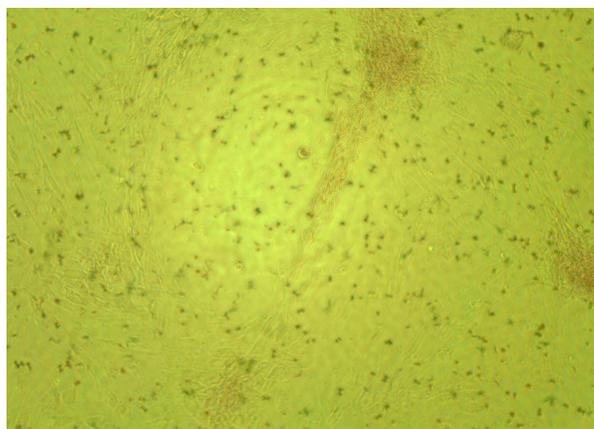


Figure 3. Non-induced ADSC



Ordering information

PT-5006	ADSCs - human adipose derived stem cells	≥1,000,000 cells
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Related products

Adipose derived stem cell growth medium

PT-4505	ADSC-GM BulletKit™	ADSC-BM (500 ml) plus SingleQuots™ of growth supplements
PT-3273	ADSC-BM	Adipose derived stem cell basal medium (500 ml)
PT-4503	ADSC-GM SingleQuots™	Formulates ADSC-BM to ADSC-GM. Contains fetal bovine serum (FBS), L-glutamine, and GA-1000.

Differentiation medium – adipogenic

PT-8002	PGM™-2 BulletKit™	Contains preadipocyte basal medium-2 (500 ml), preadipocyte growth medium-2 SingleQuots™ kit
PT-8202	Preadipocyte basal medium-2	500 ml
PT-9502	Preadipocyte growth medium-2 SingleQuots™ kit	Frozen supplements and growth factors. (fetal bovine serum, L-glutamine, GA-1000, h-insulin (recombinant), IBMX, indomethacin, dexamethasone)
PT-7009	AdipoRed™ assay reagent	
00193339	AdipoLyze™ lipolysis assay	