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Poietics™ human ADSCs – osteogenesis 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 osteoblasts, cells should be first thawed and plated onto tissue culture flasks for **at least 1** passage prior to plating cells for assays of osteogenesis.
2. It is recommended to follow the ADSC 'Instructions for use' for thawing and subculturing the cells. When ready to plate cells for osteogenic differentiation follow the section 'Osteogenic assay procedure' below.
3. It is recommended to pre-coat multi-well plates with rat tail collagen I at 4 ug/cm^2 prior to plating cells for osteogenic differentiation. This will prevent the ADSC monolayer from peeling (de-lamination), a characteristic of osteogenic differentiation.

Preparation of medium

Osteogenic induction medium

1. Decontaminate the external surfaces of the hMSC differentiation basal medium – osteogenic and the following SingleQuots™ with 70% v/v ethanol or isopropanol:
    a. Dexamethasone
    b. L-Glutamine
    c. Ascorbate
    d. Pen/Strep
    e. MCGS
2. Aseptically open the above SingleQuots™ and add the contents to the 170 ml of hMSC differentiation basal medium – osteogenic.
3. Rinse each SingleQuots™ vial with the medium. It may not be possible to recover the entire contents of each SingleQuots™. Small losses should not affect the cell characteristics.
4. Store the supplemented osteogenic differentiation medium at 2℃ to 8℃ in the dark until needed.
5. Use this media for inducing differentiation of ADSCs as directed below.

Osteogenic assay procedure

Plating cells for osteogenesis

1. For assays of osteogenic differentiation, the recommended seeding density for human adipose derived stem cells is 10,000 cells/well in a 24-well plate in 1 ml of ADSC-GM.
2. Prepare a cell suspension in ADSC-GM using the recommended seeding density and volume of media per cm^2 above.
3. Plate cells in the desired multi-well plate.
4. Incubate at 37℃, 5% CO_2 and 90% humidity.
5. The next day after cells have attached (~18-24 hours later), induce differentiation following the instructions below.

Induction of osteogenic differentiation

Osteogenic induction medium (hMSC-osteogenic) should be used once the ADSC have attached to the cell culture surface (~18-24 hours after initial seeding).

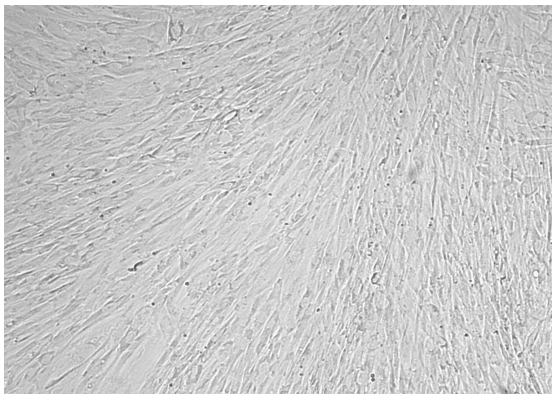
1. Carefully remove all ADSC growth medium (ADSC-GM™) from each well.
2. To cells that will remain as non-induced controls add fresh ADSC-GM™ at 1.0 ml per well in a 24-well plate.
3. To cells that will be induced for osteogenic differentiation add 1 ml of osteogenic induction medium to each well.
4. Do not let the cells dry out when changing medium.
5. Incubate at 37℃, 5% CO_2 and 90% humidity.
6. Feed the induced ADSCs every 3-4 days for 3-4 weeks by completely replacing the medium with fresh osteogenesis induction medium. Feed

non-induced control ADSCs with ADSC-GM™ on the same schedule.

7. Osteogenic induced cells will show changes in cell morphology, from spindle shaped to cuboidal shaped, as they differentiate and mineralize (see figure 1). Gaps may form in the post confluent cell layer and cells may begin to delaminate from culture surface. If this de-lamination is observed, proceed immediately to analysis of osteogenic differentiation as indicated by calcium deposition, or use the induced cells for other assays requiring osteocytes.
8. To prevent de-lamination, pre-coat tissue culture surfaces with rat tail collagen I, or equivalent, at 4 ug/cm^2.
9. For microscopic and quantitative assessment of mineralization, it is recommended to stain cultures with the OsteoImage™ mineralization assay (see figure 2).

Figure 1. Osteogenic induction of ADSC –day 21 phase contrast

a. Control



b. Induced

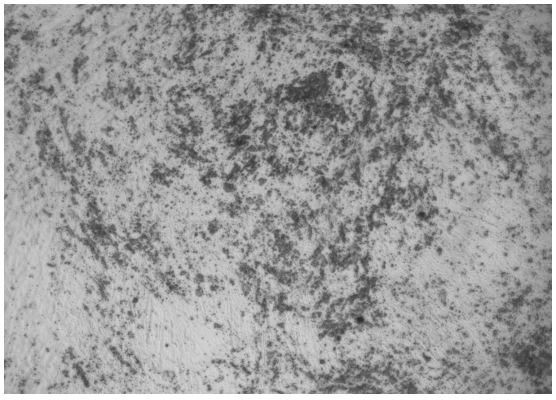
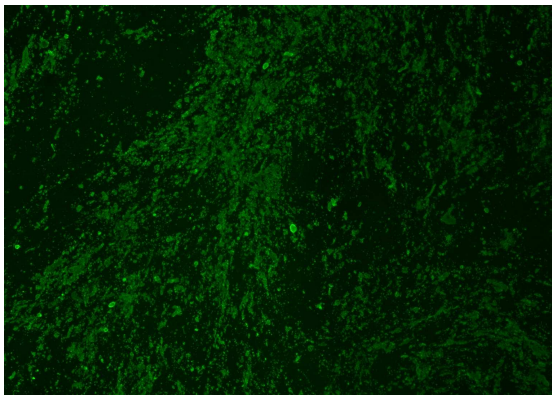


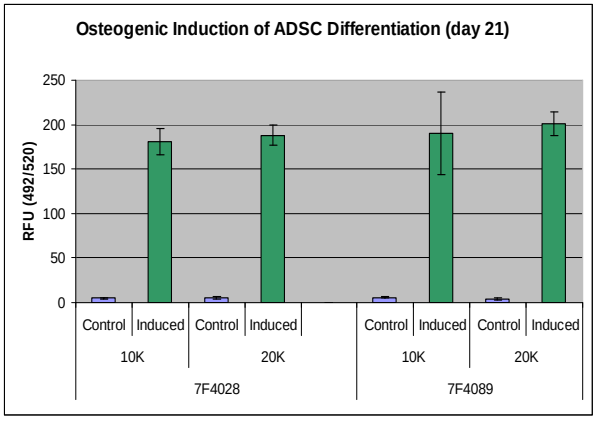
Figure 2. Osteogenic induction of ADSC – Day 21 OsteoImage™ mineralization assay

a. Control – staining results

b. Induced – staining results



c. OsteoImage™ assay quantitative results



Ordering information

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

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 media BulletKit™ – osteogenic

PT-3002	hMSC differentiation BulletKit™ – osteogenic	Contains differentiation basal medium – osteogenic (170 ml), and hMSC osteogenic SingleQuots™.
PT-3924	Osteogenic basal medium	170 ml
PT-4120	hMSC osteogenic SingleQuots™	Supplements and growth factors (dexamethasone, ascorbate, mesenchymal cell growth supplement (MCGS), L-glutamine, penicillin/streptomycin, β-glycerophosphate)
PA-1503	OsteoImage™ mineralization assay	