www.lonza.com
U.S. Scientific Support: 800-521-0390
scientific.support@lonza.com
EU/ROW Scientific Support: +49-221-99199-400
scientific.support.eu@lonza.com
Document # P-PT-5006-1 07/11


# Poietics™ human ADSCs -chondrogenesis 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 chondrocytes, cells should be first thawed and plated onto tissue culture flasks for **at least one** passage prior to plating cells for assays of chondrogenesis.
2. It is recommended to follow the ADSC 'Instructions for use' for thawing and subculturing the cells. When ready to plate cells for chondrogenic differentiation follow the section 'Chondrogenic assay procedure' below.

## Preparation of medium

### Prepare incomplete chondrogenic induction medium

1. Decontaminate the external surfaces of the hMSC differentiation basal medium – chondrogenic and the following SingleQuots™ with 70% v/v ethanol or isopropanol:
    a. dexamethasone
    b. ascorbate
    c. ITS + supplement
    d. GA-1000
    e. sodium pyruvate
    f. proline
    g. L-glutamine
2. Aseptically open the above SingleQuots™ and add the contents to the 185 ml of hMSC differentiation basal medium – chondrogenic to prepare the **incomplete** chondrogenic induction medium.
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 incomplete chondrogenic induction medium at 2°C to 8°C in the dark until needed.
5. Use this media for inducing differentiation of ADSCs as directed below.

### Prepare and aliquot TGF-β3

1. Resuspend the lyophilized TGF-β3 (Lonza PT-4124) with sterile 4mM HCl supplemented with 1 mg/ml BSA or HSA to a concentration of 20 µg/ml. For example, use 100 µl diluent for 2µg of TGF-β3.

**NOTE:** Each µl of TGF-β3 will convert 2 ml of incomplete chondrogenic medium into complete medium.

2. Aliquot small volumes of TGF-β3 into freezer-safe tubes and store at less than -70°C for no more than 6 months. (For example, the TGF-β3 can be frozen in 5 µl aliquots. Each aliquot will be sufficient to supplement 10 ml of incomplete chondrogenic induction medium.)

### Complete chondrogenic induction medium

1. After thawing, the aliquot of TGF-β3 may be centrifuged briefly at low speed to pull the small volume (e.g. 5 µl) to the bottom of the tube.
2. Pipette the volume of incomplete chondrogenic induction medium that you intend to supplement (e.g. 10 ml) into a tube.
3. To recover the full volume of TGF-β3, transfer 100 µl of this incomplete chondrogenic medium to the tube of TGF-β3.
4. Mix the solution by pipetting and transfer it back to the tube of chondrogenic induction medium. Repeat this process to be certain that you have recovered the TGF-β3. Cap and invert several times to mix.
5. The chondrogenic induction medium is now **complete**, and contains TGF-β3 at a final concentration of 10 ng/ml.

**NOTE:** Complete chondrogenic medium must be prepared fresh and used within 12 hours.

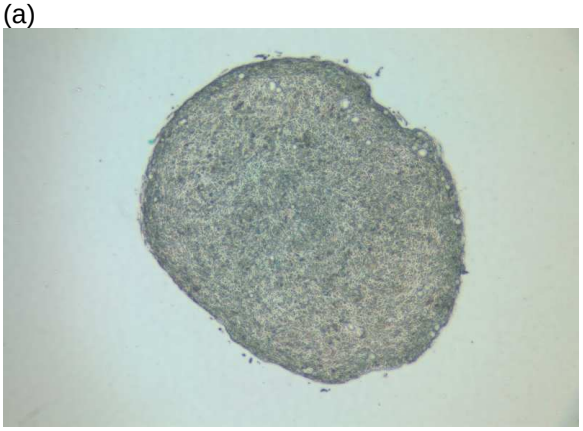
**Chondrogenesis culture protocol:**

1. Harvest ADSCs by trypisinization according to ADSC 'Instructions for use'.
2. Calculate the total number of pellet cultures required for your experiment (5.0 x $10^5$ ADSCs are needed to form each chondrocyte pellet). Transfer this amount of cells to an appropriate culture tube to wash the cells.
3. Wash the ADSCs with **incomplete** chondrogenic medium: Centrifuge the cells at 150 x g for 5 minutes at room temperature and aspirate/discard the supernatant. Resuspend the cells in 1 ml **incomplete** chondrogenic medium per 1.0 x $10^6$ cells, centrifuge again at 150 x g for 5 minutes and aspirate/discard the medium.
4. Resuspend the ADSCs in **complete** chondrogenic medium to a concentration of 5.0 x $10^5$ cells per ml.
5. Aliquot 1.0 ml (5.0 x $10^5$ cells) of the cell suspension into 15 ml polypropylene culture tubes. Centrifuge the cells at 150 x g for 5 minutes at room temperature. DO NOT aspirate the supernatant or resuspend the pellet.

   **NOTE:** Polypropylene tubes are used so that the cells do not adhere to the tube. Polystyrene tubes should not be used.

6. Loosen the cap of the tubes one half turn to allow gas exchange and incubate the tubes at 37°C, in a humidified atmosphere of 5% $CO_2$. Do not disturb the pellets for 48-72 hours.
7. Feed the cell pellets every 2-3 days by completely replacing the medium in each tube (to avoid aspirating the pellets when aspirating the medium, attach a sterile 1-200 µl pipette tip to the end of the aspirating pipette). Add 0.5 ml of freshly prepared complete chondrogenic medium to each tube.
8. After replacing the medium, gently flick the bottom of each tube to ensure the pellet is free-floating, loosen the caps and return to the 37℃ incubator.
9. Chondrogenic pellets should be harvested after at least 21 days in culture. Pellets may be formalin fixed and paraffin embedded for histological processing or may be prepared for frozen sectioning. Thin sections may be slide-mounted and stained for glycoproteins with Alcian blue. (See Figure 1)

(a)



(b)

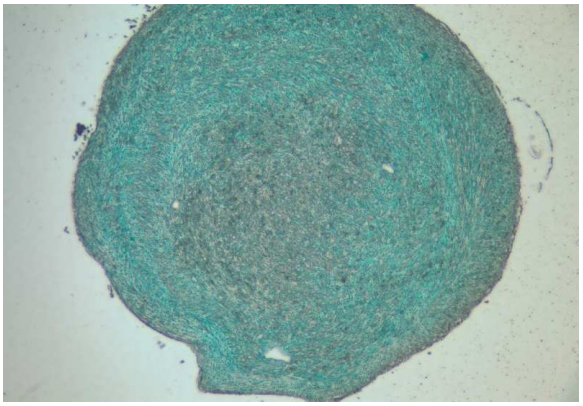


Figure 1: Alcian blue stain (a) shows ADSC cell pellet treated with **incomplete** TGF-β3 free medium. (b) shows ADSC cell pellet treated with **complete** TGF-β3 medium.

## 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™ – chondrogenic

| | | |
|---|---|---|
| PT-3003 | hMSC differentiation BulletKit™ – chondrogenic | Contains differentiation basal medium – chondrogenic (185ml), and hMSC chondrogenic SingleQuots™. |
| PT-3925 | Chondrogenic basal medium | 185 ml |
| PT-4121 | hMSC chondrogenic SingleQuots™ | Supplements and growth factors (ITS + supplement, dexamethasone, ascorbate, sodium pyruvate, proline, GA-1000, L-glutamine) |
| PT-4124 | TGF-β3 | 1 vial of lyophilized TGF-β3, 2 µg *(required chondrogenic differentiation reagent)* |

**NOTE:** TGF-β3 is supplied as a separate part from the hMSC chondrogenic SingleQuots™ kit

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Poietics™ products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* procedures.

**WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS.** Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing can not offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. If you require further information, please contact your site safety officer or Scientific Support.