Adipose-Derived MSC Isolation

This document describes the recommended protocol for isolation of mesenchymal stem cells from subcutaneous adipose tissue, using one of the new CnT-Prime MSC media. For the recommended thawing, passaging, and freezing protocols, please see the General Cultivation Protocol in the resources section of www.cellntec.com.

General Notes

This protocol describes the isolation of adMSC from subcutaneous adipose tissue, using collagenase 1 (for example Worthington cat#LS004194), and erythrocyte lysis buffer (for example Biolegend cat# 420301).

The quantities described in this protocol are for 3 grams of adipose tissue. For isolation from larger amounts, scale up the reagents accordingly.

Recommended culture media for maximum isolation efficiency are #CnT-PR-MSC or #CnT-PR-MSC-XF (referred to in the protocol as CnT-Prime MSC Medium)

At the end of the first expansion passage, cell yield is commonly in the range of 3-4 million cells.

Preparation

Before starting to work with the cells, ensure that the amount of medium, buffers and enzymes required have been pre-warmed at least to room temperature.

Always use sterile instruments, aseptic technique, and work in a laminar flow to maintain sterility.

Isolation Procedure From Adult Subcutaneous Adipose Tissue

1. For maximum cell yield, store the tissue biopsy in #CnT-XP3 biopsy maintainence medium at 4°C from sampling right up until the start of isolation.
2. Place the adipose tissue into a Petri dish, wash off any blood, and cut out the 3 gram sample using a scalpel.
3. Mince the tissue piece thoroughly with a scalple, until it is a fully homogenized slurry (5-10 mins).
4. Transfer the homogenized cell slurry into 10 mL of filter sterilized collagenase 1 solution (2 mg/mL), containing 1x antibiotics (#CnT-GAB10 for maximum heat and pH stability)
5. Incubate for 1.5 hours at 37°C, with gentle vortexing every 30 minutes.
6. Dilute the enzyme solution with 10 mL of CnT-Prime MSC medium, and allow to sit until the fat layer separates. Remove the lower clear solution with a pipette, and filter sequentially through 100 um and 70 um filters.
7. Centifuge the filtered solution at 200g for 5 minutes, and discard the surface lipid layer and supernatant using a pipette.
8. Resuspend the pellet (stromal vascular fraction) in 1 mL of CnT-Prime MSC medium, then add 3 mL of erythrocyte lysis buffer, and incubate at room temperature for 5-10 minutes (depending on the quantity of RBCs).

9. Add 10 mL of CnT-Prime MSC medium to the lysis buffer, and centrifuge at 200g for 5 minutes. Remove the supernatant, resuspend the cell pellet in 1 mL of medium, and count the cells
10. Seed cells at 20,000 cells per sq cm in CnT-Prime MSC medium with 1x antibiotics, and culture overnight.
11. Perform a medium change the following day, and then every alternate day (Mon/Wed/Fri) until cells reach confluency (typically 6-7 days).
12. Passage the cells just prior to full confluency, re-seeding at 2,000 cells per sq cm. For further culture after passaging it is recommended to omit the antibiotics.

For more information, please email out scientists directly: scientist@cellntec.com