MSC Adipogenic Differentiation

This document describes the expansion, adipogenic differentiation and Oil Red O staining of primary human mesenchymal stem cells (MSCs) grown in a CELLnTEC MSC expansion medium.

The following reagents are required for differentiation and staining:

- CnT-Prime MSC expansion medium (e.g. CnT-PR-MSC or CnT-PR-MSC-XF)
- CnT-MSCDIFF-AD.S: Adipose differentiation supplement (1 mL)
- MSC, adipose tissue-derived or bone marrow-derived
- 10% formalin
- PBS (Ca^{2+} and Mg^{2+} Free)
- 60% Isopropanol
- Oil Red O stock solution (Sigma product #O0625, 150 mg in 50 mL of 100% isopropanol)
- Haematoxylin staining solution

General Notes

Some key factors for successful expansion and differentiation of MSCs are as follows:

- Reproducible differentiation is routinely obtained up to passage 4.
- The passaging protocol during routine expansion (prior to differentiation) is an important consideration. Please follow the seeding density recommended for the expansion medium you are using, and ensure cells are passaged before reaching 100% confluency. Acctuase is recommended for detaching cells, because it is much gentler than trypsin, does not require a separate reagent to stop the reaction (simply dilute with medium immediately after detachment), and has a larger safe timing window between effective cell detachment and the point where cells are over-digested and damaged. Accutase is heat senstive, and must be stored at 4°C. It does not have to be pre-warmed to detach cells. Exposure to higher temperatures or repeated warming / cooling cycles may lead to reduced activity.
- During fixation and staining, ensure that the cells are not allowed to dry out at any point.

Thawing and Cell Expansion

1. Prepare medium: add the appropriate amount of the desired CnT MSC expansion medium to the culture flasks in which the MSCs will be expanded. Allow medium to equilibrate pH and temperature for at least 30 min at 37°C in the 5% CO_2 incubator.
2. Thaw vial of MSCs with gentle swirling in water bath at 37°C until just melted. Vials must be thawed rapidly, whilst ensuring that the temperature of the cells inside does not rise above 4°C (remove vial from water bath whilst a couple of last ice crystals remain). For more explanation about the features of an optimal thawing procedure, please review the knowledge base article in the resources section of www.cellntec.com.
3. Resuspend the cells in the cryo vial by pipetting up and down two to three times and transfer them to

the equilibrated cell culture vessels. The DMSO content must be diluted to less than 1%.

4. The volume of cell suspension seeded per vessel should deliver at least the recommended seeding density for the proliferation medium selected (2'000 cells per cm^2). Complete a medium change 6 to 24 h after seeding to remove the residual DMSO from the freezing medium.
5. Expand the cells for the desired number of passages at 37°C and 5% CO_2 with 3 medium changes per week (Mon/Wed/Fri). For best expansion and differentiation, cultures should be maintained without antibiotics / antimycotics. Passage the cells prior to reaching full confluency using Accutase (#CnT-Accutase-100) according to the general cultivation protocol available at www.cellntec.com/products/resources/protocols.

Differentiation and Staining

1. After the desired number of expansion passages, seed the cells to be differentiated at approximately 5'000 cells per cm^2 in expansion medium.
2. The next day, change medium to the desired expansion medium supplemented with the adipogenic differentiation supplement (#CnT-MSCDIFF-AD.S; 1 ml per 50 ml medium).
3. Continue to culture the cells in differentiation medium for 10-21 days, with medium changes 3x per week.
4. After the differentiation period, remove medium, wash the cells with PBS, and fix with 10% formalin solution for 45 minutes at room temperature.
5. Prepare the Oil Red O stain solution by mixing 3 parts of the stock solution with 2 parts of de-ionized water, and allow to sit for 10 minutes. Filter through Whatman no. 1 filter paper or 0.2 um filter prior to use. Use wtihin 2 hours.
6. Remove formalin solution from cells, and rinse cells with de-ionized water. Add 60% isopropanol (250 uL per cm^2) to the cells, and allow to sit for 5 minutes at room temperature.
7. Remove the isopropanol and add Oil Red O stain solution (250 uL per cm^2) to the cells, and ensure the stain is spread evenly through the culture vessel. Allow cells to inubate for 15 minutes at room temperature in the dark.
8. Remove Oil Red O solution and rinse cells twice with de-ionized water. Add haematoxylin (100 uL per cm^2) and allow to counter stain for 3 minutes. Remove the stain, and carefully rinse with running water, ensuring that the water stream does not fall directly onto the cell layer.
9. Add distilled water (250 uL per cm^2), and the cells are ready for imaging. Lipid droplets will appear red, and nucleii blue.

Further questions? CELLnTEC scientists work regularly with these cultures, and are available in case further questions arise. To contact them directly, please email scientist@cellntec.com