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MSC Chondrogenic Differentiation

This document describes the expansion and chondrogenic differentiation 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-CHOND.S: Chondrogenic differentiation supplement (1 mL)
- MSC, adipose tissue-derived or bone marrow-derived
- 4% formalin
- PBS (Ca^{2+} and Mg^{2+} Free)

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 proliferation 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 MSC 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

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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, add 250'000 cells in 500 ul proliferation medium per 15 ml tube.
2. Centrifuge at 400 g and incubate the cell pellets. Make sure the lid is loose to allow for gas exchange.
3. The next day, change medium to the desired proliferation medium supplemented with the chondrogenic differentiation supplement (#CnT-MSCDIFF-CHOND.S; 1 ml per 50 ml medium).
4. Continue to culture the cells in the differentiation medium for 21 days, with medium changes 3x per week.
5. After the differentiation period, remove medium, wash the cells with PBS, and then fix with 4% formalin solution over night at 4 °C
6. Remove formalin and wash with PBS.
7. Process samples for histology
8. Stain samples, e.g. H&E, Alcian Blue, Safranin O or IHC stain

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