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Growing SCSR Exfoliated Cells in Culture- Procedure for 6 well plates

Growth medium: Two growth media have been used for culturing exfoliated cells:

1. Mesencult™ basal medium for human mesenchymal stem cell (STEMCELL TECHNOLOGIES INC., Cat. No. 05401) mixed with mesenchymal stem cell stimulatory supplements (STEMCELL TECHNOLOGIES INC., Cat. No. 05402)

2. Methocult™ SF^{BIT}H4436 (STEMCELL TECHNOLOGIES INC., Cat. No. 04436)

Procedure for culturing colonocytes:

1. Prepare 1-5 million exfoliated cells (2-5µm Coulter counter window) using SCSR isolation kit. Frozen cells must be washed 2 times with PBS to remove freezing medium.

2. Pass the cell suspension through an 18 gauge needle 8-10 times in order to break-up mucous and prevent clumping of cells.

3. Centrifuge the culture at 2000 rpm for 10 min. and suspend the cell pellet in 2 ml of either growth medium and aspirate cells by gentle pipetting.

4. Transfer the cells to a 6 well plate.

5. Incubate cultures at 37°C and 5 % CO_2.

Medium Renewal and Subculturing:

Once per week. Subculture ratio 1:2 to 1:3

Cryoprotectant Medium:

Cell freezing medium-serum-free, Sigma Cat. No. C2639

Notes:

1. Bacteria grow along with these cultures, which seems to be healthy for exfoliated cells, unlike other cell types. Therefore to avoid contamination of other cell lines with bacteria it is recommended to grow the exfoliated cell cultures in a separate incubator.

2. During the course of the first 21 days, the culture will have significant bacterial growth. After this period, bacterial growth can be inhibited using antibiotics, if desired.