ISOLATION OF STROMAL VASCULAR FRACTION (SVF) CELLS FROM RAT ADIPOSE TISSUE

Enzymes

COL H recombinant collagenase class II ACTIVITY ≥30.0 Units/mg (Pz Grassmann)

Preparation stock solutions

1. Solution A: Reconstitute COL H, do not exceed 300 U/ml final concentration. Make aliquots of COL H 300 U total and store at -20°C

2. Solution B: dissolve thermolysin in in H2O and filter for sterility, final concentration of 1mg/ml. Make aliquots of Thermolysin 100 µg and store at -20°C

ISOLATION OF SVF CELLS FROM 1 Gr of adipose tissue

DIGESTION SOLUTION FOR 1 gr OF ADIPOSE TISSUE:
Solubilize one aliquot of Solution A in 10 ml DMEM without serum (containing Ca++ ≥ 2 mM [final concentration]) and put it on ice. Immediately before use add one aliquot Solution B.

1. Transfer 1gr of adipose tissue in 50 ml tube containing 10 ml of DIGESTION SOLUTION and incubate at 37°C for two hrs with gentle agitation.
2. Add 30 ml DMEM with 10% FBS to quench the collagenases activity .
3. Centrifuge at 300g, 10 min. r.t.
4. Discard the supernatant and resumed the pellet in 30 ml DMEM 10% FBS, to wash the cells.
5. Centrifuge at 300*g*,10 min. r.t.
6. Discard the supernatant and resumed the pellet in 10 ml DMEM with 10% FBS. Count the cells and seed into culture dish in DMEM 10% FBS plus Penicillin-Streptomycin (normally we obtain 1-2 x 10^6 of cell from 1 gr of tissue).

***Note**: This protocol is meant to be a starting point; all isolation procedures require an individual optimization.COL G and COL H concentration, protease addition and digestion time can be experimentally adjusted.*