



MSCs FROM ADIPOSE TISSUE - ISOLATION KIT

Enzymes

COL H (900 U) recombinant collagenase class II + Thermolysin (300 µg)

Stock solutions preparation

1. Dissolve COL H in 1.5 ml sterile H₂O and make 3 aliquots of 500 µl (each aliquot is the Solution A) and store at -20°C.
2. Dissolve the Thermolysin in 300 µl sterile H₂O and make 3 aliquots of 100 µl (each aliquot is the Solution B) and store at -20°C.

Isolation of MSCs from 1 gr adipose tissue

DIGESTION SOLUTION FOR 1 gr OF ADIPOSE TISSUE:

Solubilize one aliquot of Solution A in 10 ml DMEM (containing Ca⁺⁺ ≥ 2 mM [final concentration]) without serum and put it on ice. Immediately before use add one aliquot of Solution B.

1. Transfer 1 gr. of adipose tissue in a 50 ml tube containing 10 ml of DIGESTION SOLUTION and incubate at 37°C for 2h 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 300g, 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.

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.