FIBROBLASTS ISOLATION FROM HUMAN SKIN

Enzymes

COL G recombinant collagenase class I ACTIVITY ≥3.0 Units/mg (Pz Grassmann)

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

Preparation of COL G and COL H stock solutions

1. Solution A: Reconstitute COL G in H2O, do not exceed 30 U/ml final concentration.

2. Solution B: Reconstitute COL H in H2O, do not exceed 300 U/ml final concentration.

3. Filter for sterility (0.22 µm) and make aliquots of Solution A and Solution B.

4. Annotate the Unit/ml value of the stock solutions and store at -20°C

Procedure

1. Transfer the tissue in a petri dish and wash with PBS (Dulbecco's Phosphate Buffered Saline Solution without Ca2+ Mg2+). Cut the skin in 0.5 x 1.0 cm2 fragments.

2. Add 5 ml of Dispase II and incubate for 3h at 37°C or overnight at 4°C.

3. Separate dermis from epidermis with sterile tweezers and transfer the dermis in another petri dish. Wash with PBS.

4. Mince the dermis and incubate the pieces in a petri dish with 15 ml of collagenases solution for about 4 h (checking regularly the dissociation proceeding) at 37°C containing 0,5 unit/ml of G and 5 unit/ml of H plus neutral protease (1 mg/ml) in PBS (Dulbecco's Phosphate Buffered Saline Solution with Ca2+ Mg2+).

5. Collect digested dermis from the petri dish and add an amount of culture medium equal to the double of the collected volume.

6. Centrifuge 300x g for 4 min. Remove the supernatant and resuspend pellet in culture medium.

7. Seed a final volume of 5 ml in T25 flask and incubate at 37°C, 5% CO2.

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

If you have any question please contact info@abielbiotech.com.