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Human skin single cell dissociation

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Protocol status: Working
We use this protocol and it's working

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Abstract

Splitting dermis from epidermis, then dissociating single cells from human skin.

1 Cut skin into thin strips, ~1.5cm wide

2 Stretch the tissue using forceps, and using an 80µm guard on a dermatome cut the top layer of skin. Place this in ice cold PBS

3 Cut a mesh of slits into the 200µm skin layer to aid enzymatic access

4 Place the skin strips in a 50ml Falcon tube in RPMI + 2U/ml Dispase II

5 Invert the tubes to ensure the dispase has sufficient access to all surfaces

6 Seal the tubes with parafilm and place in a 37^{o}C water bath for 1 hour. Invert the tubes every 20 minutes to aid digestion

7 Dunk the skin sheets in PBS to dilute the dispase

8 Use fine forceps to peel the epidermis from the dermis, placing each in PBS in separate containers on ice while peeling

9 Place the epidermis in a cell culture dish with 20ml RF10 (RPMI + 10% FCS (v/v) + 100U/ml Penicillin + 100µg/ml Streptomycin + 1% L-Glutamine (v/v). Add 1.6mg/ml type IV Collagenase. Place this in a 37^{o}C 5% CO_2 incubator overnight (~12 hours)

10 Repeat step 9 with the dermis

11 Collect the supernatant with a serological pipette and pass it through a 100µm cell strainer, keeping the dermis and epidermis separate

12 Wash the base of the culture dish with RF10 to collect any settled cells and pass this solution through a 100µm cell strainer

13 Centrifuge the collected cells at 500g for 5 minutes (high acceleration/deceleration) and resuspend in PBS to count