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

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Single cell dissociation of healthy paediatric skin

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Human Cell Atlas Method Development Community

Emily Stephenson

DISCLAIMER

This protocol has been tested on a variety of skin sites such as lip and trunk, however not all body sites has been tested. This protocol has also only been used for healthy, non-diseased skin.

ABSTRACT

This protocol outlines the method for the enzymatic dissociation of healthy paediatric skin >3mm into a single cell suspension.

GUIDELINES

This protocol includes an overnight incubation step.

MATERIALS

Petri dish
Scalpel
Forceps
100 micron fiilters
RPMI
RF-10 (RPMI plus 10%FCS, 1% Pen-strep and 1% L-glut)
48-well v bottom plate
PBS
Dispase (Roche)
Collagenase Type IV (Worthington)
50ml Falcon Tubes
Flow Buffer (PBS 2% FCS and 2mM EDTA)

SAFETY WARNINGS

This protocol uses sharp objects.

BEFORE START INSTRUCTIONS

Do not forget to record the metadata for this sample. Before starting, clean MSC Class II with 70% ethanol and make up virkon. Ensure you have all materials needed to carry out the protocol.

Day 1 - Begin protocol late afternoon

1 Record sample meta data and assess size of sample Sample 10m

1.1 If sample is <3mmx3mm freeze and embed sample in OCT (see other protocol), if sample is >3mmx3mm continue with protocol

2 Empty sample onto petri dish and wash sample with PBS 5m

3 Cut off lower dermis and fat and place in well of 48-well plate with 1ml RPMI 2m

4 Place epidermis and upper dermis sample in a 48 well V-bottom plate with 1ml RPMI and 20µL Dispase 5m

5 Add parafilm to plate and leave in 4°C fridge overnight 1m

Day 2 - Begin protocol early in the morning

4h 8m

6 Take plate out of fridge and empty epidermis/upper dermis onto new petri dish, remove collagenase type IV from -80 freezer — 2m

7 Using forceps, separate epidermis and upper dermis and place each separately into new wells of the 48-well plate — 10m

8 Place 1ml RPMI in each well of plate containing epidermis and upper dermis — 5m

9 Add collagenase type IV 1:100 (10µL) to each of the tissues (epidermis, upper dermis and lower dermis) — 5m

10 Place in incubator and incubate at 37°C for 3 hours — 3h

11 Remove from incubator and, using 1ml pipette, pipette each tissue up and down to ensure the tissue has dissociated — 10m

12 Pipette through 100micron filter into separate 50ml Falcon tubes — 5m

13 Wash out each well an additional 3 times with 1ml RF-10 and pass through filter — 2m

14 Wash filter with 25ml RF-10 and adjust so each Falcon tube has the same volume 2m

15 Centrifuge at 500g for 5 mins at 4deg (9acc/dec) 500 x g, 4°C 5m

16 Pour off supernatant 2m

17 Resuspend pellet with 1ml of Flow Buffer 5m

18 Take 10µL for cell count and count using Trypan Blue and C-Chip Haemocytometer, record number of cells isolated for each tissue 15m

18.1 If sorting for single cell RNA seq, continue with antibody staining and FACS protocol or if freezing down cells, continue with viable cell freezing protocol