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Human adult generic tissue dissociation *in development*

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Human Cell Atlas Method Development Community

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ABSTRACT

The aim with this protocol is to pull together what we have learnt from dissociating other tissues, to generate a 'generic' protocol that can be used on multiple tissues for single cell RNA sequencing. To date (June 2019) we have mainly used this on human adult lung and gut and it is definitely still in development! We are also testing replacing liberase TL with liberase DH. The trypsin step may or may not be necessary depending on tissue, but can liberate additional cells compared to the liberase alone.

MATERIALS TEXT

Equipment
Rocker
Centrifuge
Nucleocounter or C-chips & trypan blue
Sterile Cell Culture Hood
Timer

Material	Supplier Info
Nuclease free water	Ambion (AM9939)
Liberase TL	Roche (5401020001)
DNaseI	Sigma (11284932001)
RPMI 1640 Medium, HEPES, no glutamine	Gibco (42401042)
FBS	Sigma (F7524-50ML)
Trypsin-EDTA 0.25%	Gibco (25200-056)
BSA	Sigma (A8412-100ML)
0.5M EDTA	ThermoFisher (15575020)
DPBS(-/-)	Gibco (14190094)
10cm plastic dish	Corning (430591)
Scalpels	Swann-Morton (MISC0011)
15ml Falcon tube	Falcon (352097)
50ml Falcon tube	Falcon (352098)
70µM cell strainer	Corning (15370801)
2ml syringe	BD Medical (10673555)
Red Blood Cell lysis buffer	ThermoFisher (00-4333-57)
Trypan Blue	ThermoFisher (11414815)

SAFETY WARNINGS

Biological Risks when working with primary cells from humans

- Cells from primary samples may contain uncharacterised adventitious agents, including blood-borne viruses. No attempt will be made to culture these agents deliberately. Correct use of PPE will drastically reduce the risks. Hepatitis B vaccination is recommended.

Chemical risks

- Most chemicals in this facility pose minimal risk to the individual, scoring low to medium on the risk assessments.
- Always wear correct PPE (which includes eye protection, nitrile gloves, thermal gloves for handling Liquid Nitrogen and appropriate labcoat) when handling any chemical.
- For more chemical information see the **COSHH** forms or **MSDS** for each chemical.
- For this particular SOP, please be particularly cautious with:

o Isopentane	
o Liberase TL	
o Trypsin-EDTA 0.25%	
DNase I	
Trypan blue	

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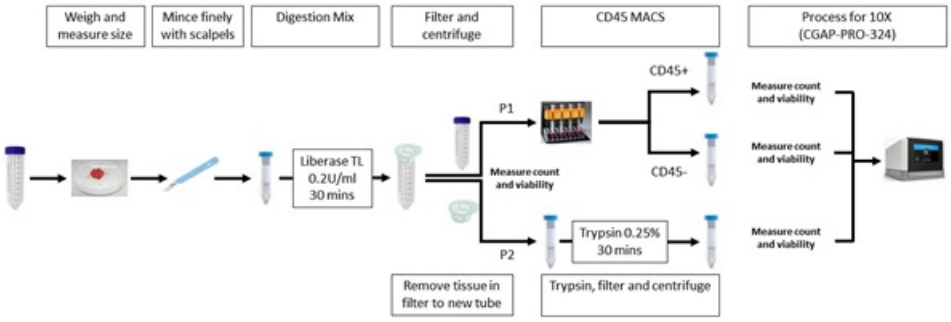

Process flow

2 **Tissue Dissociation Part 1**

Place tissue piece on a 10cm petri dish and weigh and record tissue weight and size.

3 Wash sample with cold DPBS(-/-) to remove most of the debris.

4 Cover sample with 250µl ice cold DPBS(-/-).

5 Using two scalpels, chop the piece as finely as possible.

6 Transfer minced sample into a 15ml Falcon tube (tilt petri dish and wash the dish with 1-2ml Digestion Mix and add to the falcon tube. Repeat the wash to collect any piece of tissue that may be left on the dish).
- **Digestion Mix (RT):** PBS + 250µg/ml(13U/ml) Liberase TL + 0.1mg/ml DNaseI

(e.g 5ml PBS + 77µl Liberase TL 0.2U/ml + 50µl DNase I (10mg/ml) per sample).

7 Incubate at 37°C with rocking for 30 minutes.Freeze tissue samples for bulk RNA & spatial transcriptomics at this stage. Just before dissociation finishes, take 10x beads out of -80°C to room temperature and -20°C reagents (EXCEPT enzyme) to room temperature. Enzyme can be placed on ice.

8 Pipette digested sample through a 70µM cell strainer into a 50ml Falcon tube.

9 Wash the filter through with 10ml Neutralization Media and rub through with a 2ml syringe plunger.
Neutralisation Media (RT): RPMI + 20% FBS (e.g. 40ml RPMI + 10ml FBS per sample).

10 Wash strainer again with 10ml Neutralization Media.

11 Centrifuge 700 rcf, 5min at 4°C. This is Sample P1 (Part 1).

12 **Tissue Dissociation Part 2**
While centrifuging, turn over cell strainer and wash contents into a new 50ml falcon tube using 10ml Trypsin-EDTA 0.25% with DNase I. Transfer to a 15ml Falcon tube and place back in 37°C incubator on rocker for 30 minutes.

13 **Tissue Dissociation Part 1 Continued**
The use of Red Blood Cell lysis is tissue specific. Please check if steps 14 and 15 are necessary for the tissue being processed.

14 Aspirate supernatant from Sample P1. Resuspend in 5ml ice cold 1x Red Blood Cell lysis buffer and transfer to a 15ml Falcon Tube. Place on ice for 5 min with periodic agitation.

15 Add 10ml 0.04% BSA and centrifuge 300 rcf, 5 min at 4°C then aspirate supernatant.

16 Resuspend pellet in 1ml 0.04% BSA and count cells on nucleocounter (requires 100ul volume) or with C-chips/trypan blue (requires 5ul cell volume).

17 Record cell count and viability in lab book as Pre-MACS P1.

18 If cell yield is at least 500,000 cells proceed to CD45 MACS (CGAP-PRO-322).

19 If cell yield is below 500,000 cells load directly on 10X.

20 **Tissue Dissociation Part 2 Continued**
After 30min incubation, pipette digested sample through a 70µM cell strainer into a 50ml Falcon tube.

21 Wash the filter through with 10ml Neutralization Media and rub through with a 2ml syringe plunger.

22 Wash strainer again with 10ml Neutralization Media.Note if any material is remaining in the cell strainer take a picture using the tablet.

23 Centrifuge 700 rcf, 5 min at 4°C then aspirate supernatant. This is Sample P2 (Part 2).

24 Resuspend pellet in 0.5ml 0.04% BSA and pass through 70µM cell strainer in a 50ml Falcon tube and wash through with a further 0.5ml.

25 Count cells on nucleocounter (requires 100ul volume) or with C-chips/trypan blue (requires 5ul cell volume).

26 Record cell count and viability in lab book as P2.

27 Place on ice until CD45 MACS is completed for sample P1 and then load on 10X together (CGAP-PRO-324).

28 Any remaining cells after 10X should be centrifuged at 300 rcf, 5min at 4°C.

29 Resuspend the pellet in KSR+10% DMSO and move to a cryovial and store in a CoolCell at -80°C overnight.