Sep 07, 2018

Human colon tissue dissociation for immune cells

DOI
dx.doi.org/10.17504/protocols.io.tbfeijn

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Protocol Citation: Kylie James 2018. Human colon tissue dissociation for immune cells. **protocols.io https://dx.doi.org/10.17504/protocols.io.tbfeijn**

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

Created: September 07, 2018

Last Modified: September 07, 2018

Protocol Integer ID: 15431

Keywords: colon, human, dissociation, immune cell, human colon tissue dissociation for immune cell, lymphocytes from human colon, human colon tissue dissociation, human colon, pure lymphocyte, lymphocyte, 10x chromium genomic, resultant population of pure lymphocyte, cell, single cell, sequencing data, evident in the single cell

Abstract

This has been adapted by Kylie James in Dr Sarah Teichmann's laboratory. Kylie has used this protocol many times for retrieval of lymphocytes from human colon (mLN, sigmoid, caecum and transverse) for 10X chromium genomics. FACS analysis shows a resultant population of pure lymphocytes with high viability. Stress signatures are evident in the single cell sequencing data.

Materials

MATERIALS

- Percoll **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**17-0891-01
- DPBS (no Ca, no Mg) **Thermofisher** **Catalog #**14190144
- DNase I recombinant **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**10104159001
- Liberase TL **Roche** **Catalog #**05 401 020 001
- DPBS (10X), no calcium, no magnesium **Thermo Fisher Scientific** **Catalog #**14200075
- UltraPure 0.5M EDTA, pH 8.0 **Thermo Fisher Scientific** **Catalog #**15575-038
- HEPES Buffer **Thermo Fisher Scientific** **Catalog #**15630-080
- RPMI 1640 Medium **Thermo Fisher Scientific** **Catalog #**11875-085

Troubleshooting

Safety warnings

Ergonomic Risks when working in this facility

- The processes undertaken by this facility results in a high risk of Ergonomic stresses.
- Remember to take regular breaks between tasks to minimise these risks, making sure to move and stretch out your limbs. Always sit at the hood with feet on the floor (or foot rest), back straight and arms as close to your sides as possible to retain good posture.
- Maintain general awareness of ergonomic stresses and reduce or avoid these stresses where possible. This could be by using aids such as Multi channel or repeat pipettes wherever possible to reduce repetitive use of hands, fingers and thumbs.

Chemical risks

- Most chemicals in this protocol 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.

Any chemicals which have specific risks and handling instructions will be outlined in the appropriate SOP method section

- Biological Risks when working with primary tissues 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. Individuals should be vaccinated against Hepatitis B

Make up the following reagents for 6 samples:

1 200ml PBS/HEPES: PBS (198ml) + 10mM HEPES (2ml)
200ml RPMI/HEPES*: RPMI (196ml) + 2%FBS (4ml) + 10mM HEPES (2ml)

120ml RPMI/HEPES/EDTA: RPMI/HEPES* (117.6ml) + 1 mM DTT (1.2ml)+ 5mM EDTA(1.2ml)
50ml RPMI/HEPES/Enzymes- RPMI/HEPES* (44.23ml) DNase (~60ug/ml; 1.57ml) + Liberase (0.42 mg/ml;4.2ml)

100ml 2%FBS/PBS (FACS buffer): PBS (98ml) + FBS (2ml)

Percol gradients:
30ml P-100 = 9 volumes Percoll (27ml) + 1 volume 10 x PBS (3ml)
30ml P-40 = 4 volumes of P-100 (12ml) + 6 volumes of PRMI (18ml)
18ml P-80 = 8 volumes of P-100 (14.4ml) + 2 volumes of PBS (5.4ml)

Notes on enzymes:
Liberase - comes as 2 × 5mg stock. dilute in sterile water to give 5mg/ml stock (use 673 ul per 8ml digestion cocktail)
DNase1 – (1550 KU size) – dilute in sterile water to give 4 KU/ml (use 252 ul per 8ml digestion cocktail).

Method

2 In a petri dish with the collection media, cut tissue into small pieces with surgical scissors. Transfer tissue pieces to a 50ml falcon containing 10ml PBS/ HEPES and gently agitate.

3 Poor tissue into a petri tissue. Transfer pieces into a new 50ml Falcon tube.

4 Add 10ml of RPMI/HEPES/EDTA. Incubate in shaker incubate for 20 min (37C, ~200rpm)

5 Vortex and aspirate S/N.

6 Repeat step 4-5.

7 Wash pieces in a petri dish with >10ml PBS/HEPES.

8 Using forceps, transfer the pieces to a fresh 50ml falcon tube containing 8ml RPMI/HEPES/Enzymes. Incubate for 30minutes at 37C, with shaking (~200 rpm).

9 Vortex, pass through a 100um yellow cell strainer on top of a fresh 50ml tube. Use the end of a 5ml syringe plunger to break up the remaining tissue through the strainer. Wash through with 10ml 2%FBS/PBS

10 Pellet cells by centrifuging for 10 minutes at 1200rpm and remove S/N. Resuspend in 5ml P-40 and transfer to a 15ml Falcon tube.

11 Gently underlay (using a 1ml pipette) with 3 ml P-80 percol

12 Spin for 20 minutes at 600xg (10C) with **no acceleration and break.**

13 Collect cells at the interface using a plastic Pasteur pipette or 1ml pipette into a new 15ml tube. Top up to 10ml with 2%FBS/PBS, centrifuge for 7min and remove s/n.

FACS staining cocktail for analysis and isolation of immune cells

14 Add 100ul of antibody cocktail (below) made in 2%FBS/PBS (or BD Brilliant Violet Staining Buffer if using more than 2 BV conjugated antibodies) directly to the samples. Mix well.

15 Incubate at room temp for 20mins.

16 Move to a FACS tube and using FACS buffer, wash out tube to collect as many cells as possible. Top up FACS tube to 2ml.

17 Spin for 5minutes and remove s/n.

18 To 500ul 2%FBS/PBS buffer, add:

	Target	Flurochrome	Manufacturer	Cat number	Volume added	diultion
	Live/dead	Zombie Aqua	Bioledgend	423101	2.5	1:200

	CD45	BV650	Bioledgend	304043	5	1:100
	CD3	FITC	Bioledgend	317305	5	1:100
	CD4	BV421	Bioledgend	344631	5	1:100
	CD8	PE-CY7	Bioledgend	344711	5	1:100
	CD19	APC-cy7	Bioledgend	302217	5	1:100
	IgD	PE Dazzle	Bioledgend	348207	5	1:100
	CD27	BV711	Bioledgend	356429	5	1:100
	HLADR	BV785	Bioledgend	307641	5	1:100
	CD14	APC	Bioledgend	367117	5	1:100
	CD11c	PE	Ebioscience	12-0116-42	5	1:100

Aim to sort 50000 of target cells into 500ml 2%FBS/PBS in eppendorfs.

Loading for 10X Chromium Genomics Single-cell RNAseq

19 After sort and back in the lab, pallet cells and resupend in 50ul PBS. Manually count 10ul cells diluted 1:1 in trypan blue to confirm cell number (we see that the FACS sorter over estimates the number of sorted cells by about 50%).

20 Resuspend at 1000cells/ul in PBS and aim to capture 5000 cells in the 10X chip.