Freezing and processing intestinal biopsies for the isolation of CD45+ leukocytes

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1 Works for me

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ABSTRACT

This protocol is designed for the freezing, thawing, and processing of human intestinal pinch biopsies for isolation of live CD45+ leukocytes and downstream applications such as flow cytometry or single-cell RNA-sequencing.

Oxford Human Cell Atlas protocol – Freezing and processing intestinal biopsies - v1.00.docx

MATERIALS TEXT

Reagents

Item	Catalog #	Company
MACS Tissue Storage Solution	130-100-008	Miltenyi Biotec
Cryostor Cs10 Cryopreservation medium	C2874	Sigma-Aldrich
RPMI-1640 media	R0883-500ml	Sigma-Aldrich
FBS	n/a	Multiple
Penicillin/Streptomycin	15140-122	Gibco
HEPES Buffer (1M)	15630-056	Gibco
Percoll	GE17-0891-01	Sigma-Aldrich
10x PBS	10649743	Fisher Scientific
DNase I	11284932001	Sigma-Aldrich
Collagenase D	11088882001	Sigma-Aldrich

Equipment

Item	Catalog #	Company
CoolCell LX cell freezing container	15552771	Fisher Scientific
gentleMACS C Tube	130-093-237	Miltenyi Biotec
Cell strainer 70 micron	352350	Falcon
Glass Pasteur pipettes	612-1701	VWR

Media

Complete media

500 ml of RPMI-1640 media

50 ml of FBS

5 ml of Penicillin/Streptavidin

5 ml of HEPES

Digestion media (per sample)

5 ml of Complete Media

100 ul Collagenase D (stock: 50 mg/ml)

25 ul DNase I (stock: 2 mg/ml)

Percoll solutions:

Stock isotonic percoll (9 parts Percoll, 1 part 10xPBS)
36 ml Percoll
4 ml 10x PBS

70% percoll (7 parts stock isotonic percoll, 3 parts 1x PBS)
7mL stock isotonic percoll
3mL 1x PBS

35% percoll (1 part 70% percoll, 1 RPMI-1640)
10mL 70% percoll
10mL RPMI-1640

Stage 1 - Collection in Endoscopy

1 Collect endoscopic biopsies into 50ml falcon tube with 5ml of cold MACS tissue storage solution.

2 Ensure all biopsies are immersed in the solution.

3 Keep on ice until ready to freeze.

4 Move onto Stage 2 as soon as possible, within 6 hours maximum.

Stage 2 - Freezing samples

5 Perform all stages in the hood.

6 Ensure that a CoolCell LX cell freezing container is available, and is defrosted to room temperature.

7 Label a cryovial for each sample, as appropriate.

8 Add 1000µl of Cs10 to cryovial.

9 Carefully remove all MACS tissue storage solution from the 50ml falcon with a pastette (transfer pipette) and P1000 pipette, taking care not to disrupt or discard the biopsies.

10 Using sterile forceps, transfer the biopsies into the Cs10-containing cryovial.

11 Place cryovials into CoolCell LX and transfer to -80 C freezer.

12 Move to Stage 3 on the next working day if possible.

Stage 3 - transfer samples to storage location

13 Remove sample from CoolCell LX and transfer to liquid nitrogen box (if for long-term storage), or to -80 C box (if planned for use in next 2 months).

Stage 4 - thawing and digesting biopsies

14 Perform all stages in the hood.

15 Warm Complete Media and Digestion Media to 37 C prior to beginning.

16 Proceed quickly to remove the biopsies from the freezing media.

17 Fill a gentleMACS C tube with 5 ml of Digestion Media.

18 Fill an appropriately labelled 15 ml conical with 9 ml of Complete Media.

19 Begin to thaw the frozen biopsy in a 37 C waterbath.

20 When the frozen biopsy begins to thaw, but haven't thawed completely, remove from the water bath and transfer to the hood.

21 Finish thawing the biopsy by adding 1 ml of Complete Media (from the 15 ml conical) dropwise.

22 Use a pastette to transfer the media and biopsies into the appropriate 15 ml conical.

23 Place a 70 micron cell strainer onto a 50 ml conical.

24 Pour the biopsies onto the strainer.

25 Wash with 20 ml of Complete Media.

26 Use sterile forceps to transfer the biopsies into a gentleMACS C tube filled with 5 ml of Digestion Media.

27 Homogenize on the gentleMACS using programme brain 01_02 (gentle).
Tissue tends to get caught in the rotor blade of the gentleMACS tube, use pastette to return to bottom of tube.

28 Place in a 37 C shaking incubator (220 rpm) for 1 h.

29 Homogenize on the gentleMACS using programme m_intestine_01.

30 Strain cells through 70µm filter into 50mL falcon tube. Pour onto filter.

31 Grind remaining tissue (use plunger from a syringe to break up any clumps on top of filter (pestle and mortar-action).
Wash any cells around blades, on filter or still in tube using Complete Media and a pastette.

32 Centrifuge at 600 g for 10 minutes at 4 °C.

33 Resuspend in 20 ml of Complete Media.

Stage 5 - Lymphocyte enrichment

34 Centrifuge at 600 g for 5 minutes at 4 °C.

35 Resuspend cell pellet in 6mL of 35% Percoll.

36 Transfer to 15mL falcon tube.

37 Underlay 3 mL of 70% Percoll solution using a glass Pasteur pipette.

38 Centrifuge at 800xg for 20 minutes (at RT) without the brake (decrease acceleration to 7).

39 Take the layer between 35% and 70% Percoll and transfer to a new 15mL falcon tube.
Before removing interphase, remove some of the top layer to avoid stromal cell contamination of mononuclear fraction.

40 Fill the tube to 15 ml with Complete Media.

41 Centrifuge at 600xg for 5 minutes.

42 Remove supernatant.

43 Resuspend cell pellet in 10mL Complete Media.

44 Count cells using trypan blue.

45 Cells are now ready for downstream applications