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🌐 CGAP Human Spleen Dissociation, Tissue Stability Study

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Human Cell Atlas Method Development Community

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

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ABSTRACT

Mechanical dissociation of human spleen tissue to single cells for use in 10X chromium scRNA-Seq preparation.

1	Material	Quantity	Supplier Info
	100mm Petri Dish	1	Corning (430591)

Cold PBS	30ml	GIBCO (14190-144)
Forceps	2	ThermoFisher UK Ltd (15232290)
Scalpel	1	Swann-Morton Ltd (0507)
DNase I (Stock solution 10mg/ml)	100ul	Sigma (11284932001)
100uM Cell Strainers	2	ThermoFisher UK Ltd (15380801)
50ml Falcon Tubes	2	Falcon (352098)
2.0ml Syringe	2	ThermoFisher UK Ltd. (10673555)
15ml Falcon Tubes	2	Falcon (352097)
Red Cell Lysis Buffer	5ml	Life Technologies Ltd. (00-4333-57)
BSA	400ul	Sigma Aldrich (A7906-10G)
0.5ml Eppendorf	1	Eppendorf (0030 108.035)
Trypan Blue	20ul	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N01-50)

- 2 Receive spleen punch in hypothermasol FRS (Sigma H4416) solution.

- 3 **Perform Freezing Human Tissue in Isopentane before beginning Dissociation Protocol, if frozen samples are also to be collected e.g. for bulk RNA / DNA extraction etc.**

- 4 Place spleen punch onto 100mm Glass Petri Dish.

- 5 Add 10ml Cold PBS.

- 6 Slice spleen punch into pieces (less than 10x10x10mm).

- 7 Transfer spleen pieces and PBS into 50ml Falcon Tube.
- 8 Add 100µl DNase I dropwise to spleen punch in PBS to a final concentration of 100µg/ml (DNase treatment is not essential for spleen dissociation).
- 9 Incubate at room temperature for 5 min.
- 10 Mash spleen through 100µM cell strainer above a 50ml Falcon Tube using a 2.0ml syringe plunger, washing through with 10ml Cold PBS.
- 11 Transfer to 15ml Falcon Tube.
- 12 Centrifuge at 500g for 5 min at 4°C.
- 13 Add 1ml Red Cell Lysis buffer to the pellet and resuspend by racking/tapping.
- 14 Place on ice 5 min with periodic agitation.

- 15 Add 9ml Cold PBS.
- 16 Centrifuge at 500g for 5 min at 4°C.
- 17 Resuspend pellet in 5ml Cold PBS with 0.04% BSA.
- 18 Filter through a 100µM cell strainer above a 50ml Falcon Tube.
- 19 Wash cell strainer through with 5 ml Cold PBS with 0.04% BSA.
- 20 Count cells using trypan blue and a C-Chip (manually).
- 21 Remove dead cells by following “MACS Live Dead Separation”.

Protocol

NAME
CGAP MACS Live Dead Separation

CREATED BY
Adam Hunter

PREVIEW

21.1

Material	Quantity	Supplier info
15 ml Falcon Tubes	3	Falcoo (3552097)
50 ml Falcon Tubes	1	Falcoo (3552098)
MACS Dead Cell Removal Kit	1	Miltenyi Biotec (130-0090-101)

Nucleases Free Water	19M	Amibion (AM9939)
LS Columns	1	Milt enyi Bot ec h (1300-0042-401)
0.5M DNALoBindEppendorff Tubes	1	Eppendorff (0030011008.0035)

Bovine Serum Albumin (BSA)	400 ul	Sigma-Aldrich Co. Ltd (A79906-10G)
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- 21.2** A single-cell suspension should have been prepared previously and cells number and viability assessed using 1:1 trypan blue dilution.
- A viability percentage below 70-80% usually justifies using this Dead Cell Removal protocol.
- 21.3** Remove required number of cells and place in a 15ml Falcon Tube.
- Required number of cells/total cells = volume required (ml).
- 21.4** Prepare 20ml 1X Binding Buffer by adding 1ml 20X Binding Buffer Stock to 19ml Nuclease Free Water.
- 21.5** Centrifuge cell suspension for 5min at 300g.
- 21.6** Remove supernatant.
- 21.7** Resuspend cell pellet in 100ul Dead Cell Removal MicroBeads per 10^7 cells.

- 21.8** Mix well and incubate for 15mins at room temperature.
- 21.9** When 5min of incubation remains, place MS column (if $<2 \times 10^8$ cells) or an LS column (if $<2 \times 10^9$ cells) on QuadroMACS Magnetic Cell Separator and run 500 μ l (MS column) or 3ml (LS column) 1X Binding Buffer through the LS column, using a waste 15ml Falcon Tube to catch the effluent.
- 21.10** When incubation is finished, add 1ml (MS column) or 3ml (LS column) 1X Binding Buffer to cells.
- 21.11** Run cell suspension through LS column on QuadroMACS Magnetic Cell Separator, using a 15ml Falcon Tube to catch effluent as the the live cell fraction.
- 21.12** When cells have passed through, run 4 x 500 μ l (MS column) or 4 x 3ml (LS column) 1X Binding Buffer through LS column on QuadroMACS Magnetic Cell Separator using the same falcon tube to catch effluent as the the live cell fraction.
- 21.13** Centrifuge cells at 500g for 5 min at 4°C. Resuspend in 0.5-1ml PBS + 0.04% BSA.
- 21.14** Count cells and viability using nucleocounter.
- 21.15** Resuspend in appropriate volume of 0.04% BSA in PBS to run in Chromium.

- 22** Dilute cells to 1×10^6 cell per ml in 0.04% BSA and proceed to 10X preparation for scRNA sequencing.
- 23** Ensure all unused tissue, equipment and tubes that have been in contact with primary tissue are placed into Virkon in sweetie jar for a minimum of 1 hour. After this time aspirate and disposing in relevant sharps or waste routes.