CGAP Human Spleen Dissociation

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

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Protocol

Step 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)

Step 2.
Receive spleen punch in solution.

Step 3.
Perform Freezing Human Tissue in Isopentane before beginning Dissociation Protocol

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

Step 5.

Add 10ml Cold PBS.

Step 6.
Slice spleen punch into small pieces (approximately 10x10x10mm).

Step 7.
Transfer spleen pieces and PBS into 50ml Falcon Tube.

Step 8.
Add 100µl DNAse I dropwise to spleen punch in PBS to a final concentration of 100µg/ml.

Step 9.
Incubate at room temperature for 5 min.

Step 10.
Mash spleen through 100µM cell strainer above a 50ml Falcon Tube using a 2.0ml syringe plunger, washing through with 10ml Cold PBS.

Step 11.
Transfer to 15ml Falcon Tube.

Step 12.
Centrifuge at 500g for 5 min at 4°C.

Step 13.
Add 1ml Red Cell Lysis buffer to the pellet and resuspend by racking/tapping.

Step 14.
Place on ice 5 min with periodic agitation.

Step 15.
Add 9ml Cold PBS.

Step 16.
Centrifuge at 500g for 5 min at 4°C.

Step 17.
Resuspend pellet in 5ml Cold PBS with 0.04% BSA (8µl BSA/ml PBS).

Step 18.
Filter through a 100µM cell strainer above a 50ml Falcon Tube.

Step 19.
Wash cell strainer through with 5 ml Cold PBS with 0.04% BSA (8µl BSA/ml PBS).

Step 20.
Count cells using trypan blue and a C-Chip or the nucleocounter.

Step 21.
If percentage of live cells is higher than 70-80%, cells can then be processed for scRNA-seq.a. If percentage of live cells is below 70-80%, remove dead cells by following “MACS Live Dead Separation”.

PROTOCOL

. CGAP MACS Live Dead Separation

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Step 21.1.
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.

Step 21.2.
Remove required number of cells and place in a 15ml Falcon Tube.
- Required number of cells/total cells = volume required (ml).

Step 21.3.
Prepare 20ml 1X Binding Buffer by adding 1ml 20X Binding Buffer Stock to 19ml Nuclease Free Water.

Step 21.4.
Centrifuge cell suspension for 5min at 300g.

Step 21.5.
Remove supernatant.

Step 21.6.
Resuspend cell pellet in 100ul Dead Cell Removal MicroBeads per 10^7 cells.

Step 21.7.
Mix well and incubate for 15mins at room temperature.

Step 21.8.
When 5min of incubation remains, place MS column (if <2x108 cells) or an LS column (if <2x109 cells) on QuadroMACS Magnetic Cell Separator and run 500µl (MS column) or 3ml (LS column) 1X Binding Buffer through the LS column, using a waste 15ml Falcon Tube to catch the effluent.

Step 21.9.
When incubation is finished, add 1ml (MS column) or 3ml (LS column) 1X Binding Buffer to cells.

Step 21.10.
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.

Step 21.11.
When cells have passed through, run 4 x 500µl (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.

Step 21.12.
Centrifuge cells at 500g for 5 min at 4°C. Resuspend in 0.5-1ml PBS + 0.04% BSA.

Step 21.13.
Count cells and viability using nucleocounter.

Step 21.14.
Resuspend in appropriate volume of 0.04% BSA in PBS to run in Chromium.

Step 21.15.

Material	Quantity	Supplier Info
15ml Falcon Tubes	3	Falcon (352097)
50ml Falcon Tubes	1	Falcon (352098)
MACS Dead Cell Removal Kit	1	Miltenyi Biotech (130-090-101)
Nuclease Free Water	19ml	Ambion (AM9939)
LS Columns	1	Miltenyi Biotech (130-042-401)
0.5ml DNA LoBind Eppendorf Tubes	1	Eppendorf (0030 108.035)
Trypan Blue	20ul	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N01-50)
PBS	10ml	GIBCO (14190-144)
Bovine Serum Albumin (BSA)	400ul	Sigma-Aldrich Co. Ltd (A7906-10G)

Step 22.
Dilute cells to $2x10^6$ cell per ml in 0.04% BSA and proceed to 10X preparation for scRNA sequencing.

Step 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.