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CGAP MACS Live Dead Separation

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Human Cell Atlas Metho...

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

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Troubleshooting

1

	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)

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.

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

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

5 Centrifuge cell suspension for 5min at 300g.

6 Remove supernatant.

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

8 Mix well and incubate for 15mins at room temperature.

9 When 5min of incubation remains, place MS column (if <2×108 cells) or an LS column (if <2×109 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.

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

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.

12 When cells have passed through, run 4 × 500µl (MS column) or 4 × 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.

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

14 Count cells and viability using nucleocounter.

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