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CGAP MACS Live Dead Separation

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Human Cell Atlas Method ...



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Protocol status: Working

We use this protocol and it's working

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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 $<2 \times 10^8$ 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.



- 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 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.
- 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.