CGAP Dead cell removal EasySep kit with The Big Easy Magnet

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Abstract

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Protocol

Step 1.

Material	Quantity	Supplier Info
PBS	30ml	GIBCO (14190-144)
FBS	160ul	Sigma (F7524-50ML)
CaCl2 (1mM)	1ul	VWR International Ltd (E506-100ML)
15ml Falcon Tubes	3	Falcon (352097)
Trypan Blue	20ul	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N01-50)
0.5ml Eppendorf	1	Eppendorf (0030 108.035)
EasySep Dead Cell Removal Kit	1	StemCell Technologies (17899)
EasySep “The Big Easy” (grey) magnet	1	StemCell Technologies (18001)
Bovine Serum Albumin (BSA)	1ml	Sigma-Aldrich Co. Ltd (A7906-10G)

Step 2.

A single-cell suspension should have been prepared previously (e.g. by enzymatic dissociation of a tissue) and cells number and viability assessed using 1:1 trypan blue dilution.a. A viability percentage below 70-80% usually justifies using this Dead Cell Removal protocol.

Step 3.

Prepare 8ml of Recommended medium (PBS (8ml) + 2% FBS (160ul) + 1mM CaCl2 (1ul)).

Step 4.

Centrifuge samples at 500g for 5 minutes.

Step 5.

Remove supernatant and resuspend in the appropriate volume of recommended medium (0.25 - 8ml) to obtain a suspension with 1 x 108 cells/ml.a. If total number of cells is below 2.5 x 107, resuspend in the

minimum volume, i.e. 0.25ml.

Step 6.
Transfer cell suspension to a 15ml Falcon.

Step 7.
Add Dead Cell Removal (Annexin V) Cocktail to sample:a. 50uL per ml of sample.

Step 8.
Add Biotin Selection Cocktail to sample:a. 50uL per ml of sample.

Step 9.
Mix (up and down with pipette) and incubate for 3 min at RT.

Step 10.
Vortex RapidSpheres™ for 30 seconds.a. Particles should appear evenly dispersed.

Step 11.
Add RapidSpheres™ to sample and mix:a. 100µL per ml of sample.b. No incubation, IMMEDIATELY move to next step.

Step 12.
Add Recommended medium to top up the sample to the indicated volume:a. Top up to 5ml for samples ≤ 2ml.b. Top up to 10ml for samples > 2ml.

Step 13.
Mix by gently pipetting up and down 2 -3 times.

Step 14.
Place the tube (without lid) into the magnet and incubate for 3 mins at RT.

Step 15.
Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring the enriched cell suspension into a new tube.a. Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Step 16.
Count cells and viability using 1:1 trypan blue dilution.

Step 17.
Add 5ml PBS with 0.04% BSA (200ul) to wash cells.

Step 18.
Centrifuge at 500g for 5 minutes.

Step 19.
Resuspend in appropriate volume of 0.04% BSA in PBS to run in Chromium.