JUL 11, 2018

CGAP Dead cell removal EasySep kit with The Big Easy Magnet

Adam Hunter¹

¹CGAP

Human Cell Atlas Method Development Community

Adam Hunter

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.qz6dx9e

Protocol Citation: Adam Hunter 2018. CGAP Dead cell removal EasySep kit with The Big Easy Magnet.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.qz6dx9e>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

Created: Jun 15, 2018

Last Modified: Jul 11, 2018

PROTOCOL integer ID:

13086

1

Supplier Info	Quantity	Material
GIBCO (141190-144)	300 ml	PBS
Sigma (F7524-50 ML)	1600 ul	FBS
VWR International Ltd (E5506-1000 ML)	1 ul	CaCl ₂ (1 mM)

15 Falcon Tubes	3	Falcon (352097)
Trypan Blue	20 ul	Fisher Scientific (11414815)
C-Chips	1	Cambridge Bioscience (DHC-N201-50)

0.5m Eppendorf	1	Eppendorf (0030011008.0035)
EasySep Dead Cell Removal Kit	1	Stem Cell Technologies (17899)
EasySep The Big Easy" (grey) magnet	1	Stem Cell Technologies (180001)

Bovine Serum Albumin (BSA)	1m	Sigma-Aldrich Co. Ltd (A79006-10G)
----------------------------	----	------------------------------------

- 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.
- 3 Prepare 8ml of Recommended medium (PBS (8ml) + 2% FBS (160ul) + 1mM CaCl₂ (1ul)).
- 4 Centrifuge samples at 500g for 5 minutes.
- 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.
- 6 Transfer cell suspension to a 15ml Falcon.
- 7 Add Dead Cell Removal (Annexin V) Cocktail to sample:a. 50uL per ml of sample.

- 8 Add Biotin Selection Cocktail to sample:a. 50uL per ml of sample.
- 9 Mix (up and down with pipette) and incubate for 3 min at RT.
- 10 Vortex RapidSpheres™ for 30 seconds.a. Particles should appear evenly dispersed.
- 11 Add RapidSpheres™ to sample and mix:a. 100μL per ml of sample.b. No incubation, IMMEDIATELY move to next step.
- 12 Add Recommended medium to top up the sample to the indicated volume:a. Top up to 5ml for samples $\leq$ 2ml.b. Top up to 10ml for samples $>$ 2ml.
- 13 Mix by gently pipetting up and down 2 -3 times.
- 14 Place the tube (without lid) into the magnet and incubate for 3 mins at RT.
- 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.

- 16** Count cells and viability using 1:1 trypan blue dilution.
- 17** Add 5ml PBS with 0.04% BSA (200ul) to wash cells.
- 18** Centrifuge at 500g for 5 minutes.
- 19** Resuspend in appropriate volume of 0.04% BSA in PBS to run in Chromium.