# CGAP MACS Live Dead Separation

**Adam Hunter**

## Abstract

**Citation:** Adam Hunter CGAP MACS Live Dead Separation. **protocols.io**
dx.doi.org/10.17504/protocols.io.qz5dx86
**Published:** 11 Jul 2018

## Protocol

### *Step 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) |

### *Step 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.

### *Step 3.*

Remove required number of cells and place in a 15ml Falcon Tube.

- Required number of cells/total cells = volume required (ml).

### *Step 4.*

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

### *Step 5.*

Centrifuge cell suspension for 5min at 300g.

***Step 6.***
Remove supernatant.

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

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

***Step 9.***
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 10.***
When incubation is finished, add 1ml (MS column) or 3ml (LS column) 1X Binding Buffer to cells.

***Step 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.

***Step 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.

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

***Step 14.***
Count cells and viability using nucleocounter.

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