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Working

Miltenyi MACS Bead Isolation 👄

PLOS One

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ABSTRACT

This protocol includes the step-by-step used for isolation of a cell popultation of interest using MACS bead technology. The kits used for this protocol were obtained from Miltenyi. Miltenyi isolation kits are based on a simple process, in which biotin conjugated antibody-labelled mononuclear cells are separated from unlabeled cells via a column in the presence of a magnetic field.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0213832

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Moore DK, Motaung B, Plessis Nd, Shabangu AN, Loxton AG, SC (2019) Isolation of B-cells using Miltenyi MACS bead isolation kits. PLoS ONE 14(3): e0213832. doi: 10.1371/journal.pone.0213832

PROTOCOL STATUS

Working

GUIDELINES

Unless otherwise stated, all steps were performed in a biosafety cabinet under sterile conditions. All reagents were stored at 4° C prior to cell isolation, and kept on ice during sample processing. As per the manufacturers instructions, all light sensitive MACS reagents were kept in the dark. Isolation equipment (Magnet stand and Magentic seperator) were stored at -20° C prior to cell isolation.

MATERIALS

NAME V	CATALOG # ~	VENDOR ~
15 ml sterile falcon tubes and rack		
1x Phosphate-Buffered Saline	04-479Q	Lonza
Trypan Blue Solution 0.4% Sterile-filtered	T8154	Sigma Aldrich
MACS Seperation Buffer	130-091-221	Miltenyi Biotec
LS Columns	130-042-401	Miltenyi Biotec
MACS MultiStand	130-042-303	Miltenyi Biotec
Miltenyi Cell isolation Kit	View	Miltenyi Biotec
STEPS MATERIALS		
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MATERIALS TEXT

With regards to Miltenyi cell isolation kits, the addition (volume) and incubation of the labelling reagents differes depending on the kit used. However, the overall isolation process remains the same. In instances where 2 labelling reagents are present, the first reagent is added, at the reccommended concentration (according to cell number), and the sample incubated for 5min. Following this the second is added, at the reccommended concentration (according to cell number), and the sample incubated for a further 10min. In instances where just one labelling reagent is present, the reagent is added at the reccommended concentration (according to cell number), and the sample incubated for a total of 15min.

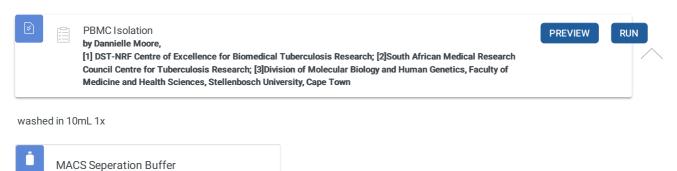
SAFETY WARNINGS

BEFORE STARTING

Prior to MACS bead isolation, samples must undergo PBMC isolation. For more detail on this pre-isolation process see dx.doi.org/10.17504/protocols.io.yfvftn6 [PROTOCOL DOI].

1 Isolated PBMCs

by Miltenyi Biotec
Catalog #: 130-091-221



1	1	In a 50ml	Falcon tube,	add 15mL o	f Ficoll-Histoplad	que Plus	media

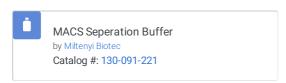
1.2 In a separate 50mL Flacon tube, dilute peripheral blood in 1:1 ratio with 1x phosphate-buffered saline to a max volume of 35mL (max starting volume of blood is 18mL)

Note: if a larger volume of blood is required - the blood must be split across seperate falcon tubes and isolated PBMCs combined following isolation procedure, prior to cell counting

- 1.3 Gently layer the diluted blood from step 3 onto the FicoII from step 2. This can be done free-hand or using a graduated pipette and pipette man
- 1.4 Once the blood has been layered and tubes secured, remove from hood and insert into benchtop centrifuge.
- 1.5 Spin at 400xg for 25 min at room temperature with the accelerator and brake OFF.
- 1.6 Once centrifuge has stopped, remove tubes and place in hood for further processing.
- 1.7 Use a sterile Pasteur pipette, carefully remove the upper plasma layer until 5cm above opaque PBMC band. In a circular motion, collect the PBMC band at the Ficoll interface and transfer into a new 50mL Falcon tube

Note: if a large volume of blood was processed and blood split into several tubes, collect and decant all PBMC bands into single falcon tube.

- 1.8 Wash isolated PBMCs twice in 50mL of PBS. Centrifuge cell suspension at 400xg for 10 min at room temperature with the brake and accelerator set to max.
- 1.9 Dilute cells in 1:10 ratio with PBS and trypan blue. Count the cells using haemocytometer and microscope. Record observed cell number and cell viability
 - PBMCs resuspended in 1x



Reccommended volume per 1x10⁷ cells according to kit specifications

3 MACS isolation labelling reagents in



added according to the manufacturers instruction (volume and incubation time). During this step, samples are incubated at 👃 4 °C

4 Following incubation, 1 ml 1x



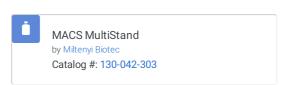
is added to cell suspension

- 5 Centrifuge cell suspension at 300xg for © 00:10:00 at 8 4 °C
- 6 Cells resuspended in 300 μl 1x

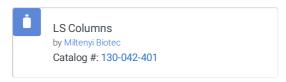


up to total cell count of 1x10⁸

7



removed from storage and set up in hood.



Placed in MACS seperator and primed with 3x = 1 ml

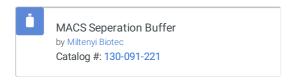


Cell suspension applied to primed LS Columns by Miltenyi Biotec Catalog #: 130-042-401 Note: Tube in which cells stored rinsed 3x with 1 ml MACS Seperation Buffer by Miltenyi Biotec

and this suspension added to the LS column (wash 1)

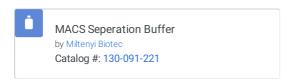
9 LS Column topped up twice with 3 ml

Catalog #: 130-091-221



(Wash 2 and 3)

- 10 Flow through collected (isolated cell population of interest during negative selection process)
- 11 In seperate tube, plunge LS Column with 3 ml

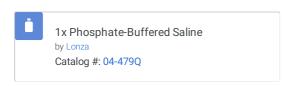


(isolated cell population of interest during positive selection process)

- 12 Centrifuge cell suspension (containing cell population of interest) at 350xg for © 00:10:00 at § 4 °C
- 13 Resuspend Isolated cells in $\frac{1}{2}$ 500 μ l $\frac{1}{2}$ ml (depending on cell type)



14 Dilute cells in 1:10 ratio with



and



Count the cells using haemocytometer and microscope. Record observed cell number and cell viability

15 Aliquot 10 μl cell suspension for sample purity check.

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