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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^{o}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^{o}C prior to cell isolation.

MATERIALS

NAME	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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Trypan Blue Solution 0.4% Sterile-filtered	T8154	Sigma Aldrich

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

PBMC Isolation
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PREVIEW RUN

washed in 10mL 1x

MACS Seperation Buffer
by Miltenyi Biotec
Catalog #: 130-091-221

at 400xg for 00:10:00 at 23 °C **(Room Temp)**

1.1 In a 50mL Falcon tube, add 15mL of Ficoll-Histoplaque 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 Ficoll 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

2 PBMCs resuspended in 1x

MACS Seperation Buffer
by Miltenyi Biotec
Catalog #: 130-091-221

Reccommended volume per 1x10^7 cells according to kit specifications

3 MACS isolation labelling reagents in

Miltenyi Cell isolation Kit
by Miltenyi Biotec
View

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

MACS Seperation Buffer
by Miltenyi Biotec
Catalog #: 130-091-221

is added to cell suspension

5 Centrifuge cell suspension at 300xg for 00:10:00 at 4 °C

6 Cells resuspended in 500 µl 1x

MACS Seperation Buffer
by Miltenyi Biotec
Catalog #: 130-091-221

up to total cell count of $1x10^8$

7

MACS MultiStand
by Miltenyi Biotec
Catalog #: 130-042-303

removed from storage and set up in hood.

LS Columns
by Miltenyi Biotec
Catalog #: 130-042-401

Placed in MACS seperator and primed with 3x 1 ml

MACS Seperation Buffer
by Miltenyi Biotec
Catalog #: 130-091-221

8 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
Catalog #: 130-091-221

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

9 LS Column topped up twice with **3 ml**

MACS Seperation Buffer
by Miltenyi Biotec
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**

MACS Seperation Buffer
by Miltenyi Biotec
Catalog #: 130-091-221

(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 **500 µl** - **2 ml** **(depending on cell type)**

MACS Seperation Buffer
by Miltenyi Biotec
Catalog #: 130-091-221

14 Dilute cells in 1:10 ratio with

1x Phosphate-Buffered Saline
by Lonza
Catalog #: 04-479Q

and

Trypan Blue Solution 0.4% Sterile-filtered
by Sigma Aldrich
Catalog #: T8154

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

15 Aliquot **10 µl** cell suspension for sample purity check.