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Miltenyi MACS Bead Isolation

PLOS One

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ABSTRACT

This protocol includes the step-by-step used for isolation of a cell population 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.

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 separator) were stored at -20°C prior to cell isolation.

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Keywords: MACS bead
Isolation, B-cell Isolation,
Miltenyi, MACS, T-cell Isolation

MATERIALS

- ✂ 15 ml sterile falcon tubes and rack **Contributed by users**
- ✂ 1x Phosphate-Buffered Saline **Lonza Catalog #04-479Q**
- ✂ Trypan Blue Solution 0.4% Sterile-filtered **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8154**
- ✂ MACS Separation Buffer **Miltenyi Biotec Catalog #130-091-221**
- ✂ LS Columns **Miltenyi Biotec Catalog #130-042-401**
- ✂ MACS MultiStand **Miltenyi Biotec Catalog #130-042-303**
- ✂ Miltenyi Cell isolation Kit **Miltenyi Biotec**

STEP MATERIALS

- ✂ MACS Separation Buffer **Miltenyi Biotec Catalog #130-091-221**
- ✂ Miltenyi Cell isolation Kit **Miltenyi Biotec**
- ✂ MACS Separation Buffer **Miltenyi Biotec Catalog #130-091-221**
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- ✂ MACS Separation Buffer **Miltenyi Biotec Catalog #130-091-221**

With regards to Miltenyi cell isolation kits, the addition (volume) and incubation of the labelling reagents differs 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 recommended concentration (according to cell number), and the sample incubated for 5min. Following this the second is added, at the recommended 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 recommended concentration (according to cell number), and the sample incubated for a total of 15min.

BEFORE START INSTRUCTIONS

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

Protocol

NAME
PBMC Isolation

CREATED BY
Dannielle DK Moore

PREVIEW

washed in 10mL 1x

MACS Separation Buffer **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-Histopaque Plus media
- 1.2 In a separate 50mL Falcon 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 separate 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 Separation Buffer **Miltenyi Biotec Catalog #130-091-221**

Recommended volume per 1×10^7 cells according to kit specifications

3 MACS isolation labelling reagents in

Miltenyi Cell isolation Kit **Miltenyi Biotec**

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 Separation Buffer **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 Separation Buffer **Miltenyi Biotec Catalog #130-091-221**
up to total cell count of 1×10^8
- 7
MACS MultiStand **Miltenyi Biotec Catalog #130-042-303**
removed from storage and set up in hood.
- LS Columns **Miltenyi Biotec Catalog #130-042-401**
Placed in MACS separator and primed with 3x 1 mL
MACS Separation Buffer **Miltenyi Biotec Catalog #130-091-221**
- 8 Cell suspension applied to primed
LS Columns **Miltenyi Biotec Catalog #130-042-401**
- Note: Tube in which cells stored rinsed 3x with 1 mL
MACS Separation Buffer **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 Separation Buffer **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 separate tube, plunge LS Column with 3 mL
MACS Separation Buffer **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 Separation Buffer **Miltenyi Biotec Catalog #130-091-221**
- 14 Dilute cells in 1:10 ratio with
1x Phosphate-Buffered Saline **Lonza Catalog #04-479Q**
and
Trypan Blue Solution 0.4% Sterile-filtered **Merck MilliporeSigma (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.