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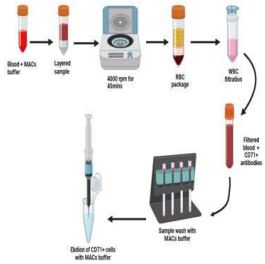
Enrichment of Reticulocytes from Whole Blood



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Human Cell Atlas

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We use this protocol and it's working

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Abstract

Reticulocytes are a group of immature RBCs produced in the bone marrow and released into the bloodstream where they mature into RBCs within one to two days. They play a crucial role in assessing bone health and many haematological diseases. Isolating viable reticulocytes for use in single-cell transcriptomics has been a challenge due to their similarity to mature RBCs. Their low concentration and continuous maturation to RBCs with time also poses a great challenge. In this study, we designed a protocol for enrichment of reticulocytes from whole blood, with a high yield and viability for single-cell transcriptomic studies.

Attachments

DOCX

Protocol for Reticul...

17KB

Guidelines

This protocol is used to enrich and obtain high concentrations of viable reticulocytes, which could be useful for single cell transcriptomics studies.

Materials

- EDTA tubes (BioRapid)
- Falcon tubes (15ml) (Corning)
- Eppendorf tubes (1.5ml) (Eppendorf)
- Acrodisc White Blood Cell filters (25mm diameter) (Cytiva)
- Ice making machine (Kojak)
- Magnetic activated cell sorting (MACS) buffer (Miltenyi Biotech)
- Ficoll paque (Cytiva)
- Swinging Bucket centrifuge (International Equipment Co. A division of Damon)
- CD71 human antibodies (Miltenyi Biotech)
- MACS column (Miltenyi Biotech)
- MACS separator (Miltenyi Biotech)
- Light microscope (Leica microsystems)
- Countess III automated cell counter (Thermo Fisher Scientific)
- Countess cell counting chamber slides (Thermo Fisher Scientific)
- New methylene blue (NMB) (Sigma Aldrich)
- 100- 1000µl Micro pipette (Agros 240-21 Omega 8)
- 0.1- 10µl Micro pipette (Agros 240-21 Omega 8)
- 100µl Pippete tips (Argos technologis)
- 10µl Pippete tips (Argos technologis)
- 1000µl Pippete tips (Argos technologis)
- Glass slides(Corning)

Safety warnings

Time is of utmost importance, as high cell viability is necessary for downstream processing of the enriched reticulocytes for single cell transcriptomics.

Ethics statement

This protocol needs prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s).

Before start

Ensure to have all materials and reagents ready before you begin. This is because reticulocytes continue to mature rapidly into RBCs, which could lead to decrease in their number and significant loss after enrichment.

Protocol

1 **Stage 1: Sample collection**

1.1 Collect 5 mL of blood sample by venepuncture in an EDTA tube.

1.2 Immediately place the sample on ice and transport to the lab for further analysis.

Move to stage 2 within two hours of sample collection.

2 **Stage 2: Reticulocyte Enrichment**

2.1 Aliquot the blood samples, 2 mL each, into separate 15 mL falcon tubes.

2.2 Dilute 2 mL blood sample with an equal volume of MACS buffer.

2.3 Into another 15ml falcon tube, layer 2 mL of ficoll and then 2 mL of the diluted blood sample above the ficoll.

2.4 Centrifuge at 4000rpm for 45mins.

2.5 With the help of a micropipette, collect the RBC package (1 mL) at the bottom of the tube, and transfer into a new 15 mL falcon tube.

2.6 Dilute the RBC package with 1 mL of MACS buffer.

2.7 Filter the diluted sample through a WBC filter (25mm diameter), to leukodeplete the sample.

2.8 Collect the flow-through into a new 15ml falcon tube.

2.9 Add 0.1 mL of CD71 antibody to 1ml of the filtered sample and incubate at room temperature for 15 minutes.

2.10 Mix the sample gently and put 1 mL of the sample into the MACS column attached to the MACS separator.

2.11 As the sample flows through the column, add 1 mL of MACS buffer to wash.

2.12 Add another 1 mL of MACS buffer for a second wash.

2.13 Add another 1 mL of MACS buffer for a third wash.

2.14 Add another 1 mL of MACS buffer for a fourth wash.

2.15 Add another 1 mL of MACS buffer for a fifth wash.

2.16 Discard the flow-through, which is mostly matured RBCs.

2.17 The reticulocytes are retained on the magnetic beads in the MACS column, due to their binding to the CD71 antibodies.

2.18 . Elute the $CD71^+$ cells (reticulocytes) using 1 mL MACS buffer, with the help of a plunger, into a new 1.5 mL Eppendorf tube.

2.19 The tubes containing the sample should always be on ice.

Move to stage 3 immediately

3 Stage 3: Visualisation and determination of cell viability

3.1 Into a new 1.5 mL Eppendorf tube, take 10 µL of the CD71^{+} sample and stain with 10 µL of New methylene blue (NMB).

3.2 Incubate for 15min at room temperature and view under the microscope.

3.3 Also, take 10 µL of the CD71^{+} sample and put into a Countess cell counting chamber slide.

3.4 Insert the chamber slide into a Countess 3 automated cell counter to count the cells and determine their percentage viability.

3.5 The cells are ready to be used for further analysis as required.