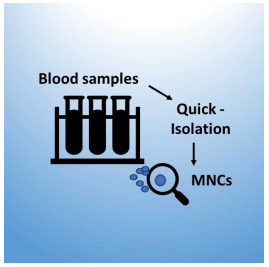
Jul 17, 2024

Simple and fast technique for separating human mononuclear cells from small amounts of blood and buffy coat with high cell yield



DOI
dx.doi.org/10.17504/protocols.io.14egn6m9zl5d/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.14egn6m9zl5d/v1

Protocol Citation: Sudhir Bhatia, Gudrun Baersch 2024. Simple and fast technique for separating human mononuclear cells from small amounts of blood and buffy coat with high cell yield . **protocols.io https://dx.doi.org/10.17504/protocols.io.14egn6m9zl5d/v1**

Manuscript citation:
Bhatia, S., & Baersch, G. (2023). Rapid Isolation of Mononuclear Cells with High Yield from Minimal Blood Volumes: A Simplified and Robust Approach for Immunotherapeutic Applications. *Medical Science and Discovery*, *10*(10), 838–841. https://doi.org/10.36472/msd.v10i10.1062

Protocol status: Working
We use this protocol and it's working

Created: July 12, 2024

Last Modified: July 17, 2024

Protocol Integer ID: 103299

Keywords: Mononuclear cell separation, Magnetic beads, Genekam MNC isolator, Cell therapies, CAR-T, ELISA, B cells, T cells, CD4, CD8

Disclaimer

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Abstract

We are again describing a rare and very simple protocol here:

The applications for human mononuclear cells (MNCs) are increasing, as these cells can be used for various tests, cell therapies and the development of human therapeutic antibodies. Density gradient isolation is still used today to separate MNCs. Diluted blood or buffy coat is added to the density gradient liquid, which is then centrifuged to obtain the different fractions. The white layer contains the MNCs, which are carefully pipetted out and washed to obtain the cells. The disadvantage of this laborious procedure is that blood and density gradient fluid may mix, which can lead to wastage of the valuable blood sample.

We have developed a simple procedure in which the user waits a few minutes after mixing the blood sample in the Quick MNC isolator in a tube. The sample is then washed two or three times with PBS and centrifuged to obtain a pellet. Although small amounts of red blood cells occasionally remain in the pellet, they have no effect on the cells in the assay, e.g. when separating with magnetic beads, or on further experiments with isolated MNC cells. A high cell yield can be achieved with a single milliliter of blood or buffy coat. This MNC isolator method is an extremely fast, simple and effective method. Even very small amounts of buffy coat or blood, e.g. 100µl, can be used to isolate MNC.

There is no documented case study in the literature using the density gradient method to separate MNC from such small amounts of blood or buffy coat. Our method is only option for small volumes.

If the user cultivates the MNC in media with fetal calf serum (FCS), it is sufficient to use 2-3% FCS instead of the usual 10% FCS. This helps to reduce the financial burden on laboratories. Please read our publication in the reference section.

Using this protocol, we have developed many cell cultures with MNC over the last 14 years.

Image Attribution

Genekam MNC isolator application

Guidelines

Ethics committee approvals should taken in consideration before starting the experiments.

Materials

-Quick MNC Isolator (Productnumber: STEM12) (Genekam Biotechnology AG, Germany)
-Sterile filter pipette tips and pipettor
-Sterile PBS (Biochrome, Germany; Lonza, USA)
-Laminar flow
-15 or 50 ml sterile tubes
-Centrifuge
-Waste collection system
-Sterile pipettes (Sarstedt, Germany)

Safety warnings

-Make some experience with this protocol before using important samples.
-Work under laminar flow to conduct cell culture experiment in order to avoid contamination.
-Waste should be autoclaved before disposing it off or treated accordingly alternatively.
-Use hand gloves and protective clothes while working with blood samples.

Ethics statement

Approval from ethics committee of your institute may be needed to conduct with this protocol. Please check this.

Before start

-Please read the protocol carefully.
-Disinfect the working place as well as surfaces of laminar flow.
-Pipette tips must be sterile.
-Laminar flow should be disinfected through UV light for 30 minutes before use.
-Waste collection system must have some disinfectant like Sodium hypochlorite or 70% ethanol etc.

A. Isolation of MNC from human peripheral blood or buffy coat: 27m

1 Add 900µl Genekam Quick MNC Isolator to each tube containing up to 100µl whole blood or buffy coat in a sterile tube (1:10). Otherwise, the ratio of solution to sample should be (1:5). For example, if you want to isolate MNC from 2 ml of buffy coat, add 8 ml of Quick MNC Isolator. A sterile 15 ml tube is sufficient for use. Alternatively, a sterile 50 ml tube can be used for larger volumes of blood. 5m

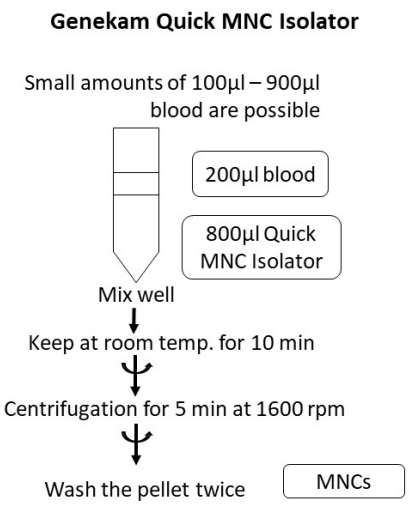
2 Work with the smallest amount of sample to gain experience with the kit instead of losing the entire sample the first time. Shake each tube gently immediately after adding the solution. Store at room temperature away from light for 10 minutes. 10m

3 Centrifuge for 5 minutes at 1600rpm. Aspirate or discard the supernatant without disturbing the pellet. 5m

4 Resuspend the pellet in the appropriate buffer (in most cases this is PBS) and wash it twice in this way.
Centrifuge each time for 5 minutes at 1600rpm and slow down the speed.
If the pellet still contains ethrocytes, treat the sample with Genekam Quick MNC isator and repeat the washing step. 5m

5 Resuspend the pellet and proceed with further procedures, e.g. counting the MNC, and perform analyses such as cell culture, magnetic separation of specific populations as well as nucleic acid isolation to perform molecular analyses. 2m

6



Protocol references

-Bhatia S. **RIBONUCLEIC ACID ISOLATION FROM HUMAN MONONUCLEAR CELL CULTURE WITH MAGNETIC BEADS PRE-ENRICHMENT FOR MOLECULAR ANALYSIS**. SANAMED. 2024. DOI: 10.5937/sanamed0-50158

-Bhatia S. 2-3% fetal calf serum in cell media is sufficient for the development and growth of human mononuclear cell cultures. Atlantic J Med Sci Res. 2023;3(2):46-9. doi: 10.5455/atjmed.2023.01.06.