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SHORT PROTOCOL No. 72

Isolation of PBMC Using Ficoll-Paque™ Plus in the new Eppendorf SpinPro® 6 R Centrifuge and Rotor S-2×Universal ID

Introduction

In this protocol we present a standardized method for PBMC isolation from "buffy coats" (whole blood concentrates without serum) using Ficoll-Paque™ PLUS [1]. The protocol was adapted for the new 2 L Eppendorf SpinPro® 6 R centrifuge equipped with the Rotor S-2× Universal ID (Fig. 1).

Peripheral blood mononuclear cells (PBMC) represent the major immune cell population circulating in peripheral blood, including lymphocytes (T cells, B cells, and NK cells) and monocytes. These cells, which are characterized by a single, round nucleus, form the basis of the innate immune system and are commonly used in immunology and clinical as well as molecular biology research. High-quality PBMC isolation is essential for reliable downstream applications and their isolation from whole blood is routinely performed by Ficoll density gradient centrifugation, developed by Bøyum in 1968 [2,3]. The Ficoll medium enables the separation of PBMC from other blood components, as PBMC will accumulate at the plasma-gradient boundary (Fig. 2).

One key factor for successful isolation of PBMC from other blood components is the formation of a well-defined interphase between plasma and the medium. Vibration during centrifugation can disrupt this interphase, leading to reduced purity and yield of PBMC. Especially the acceleration and deceleration phases can be critical, as they are typically associated with increased vibration. Therefore, it is essential not only to use a centrifuge that operates with low vibration at constant speed, but also one that offers adjustable acceleration and deceleration rates to minimize vibrations during these critical phases of the separation process.

Figure 1: SpinPro® 6 R with Rotor S-2×Universal ID.

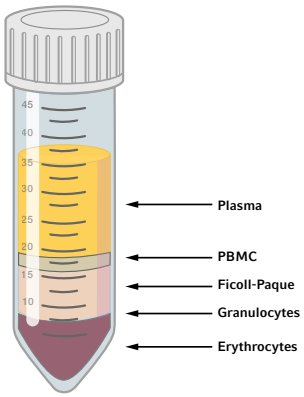

Figure 2: Density gradient of a buffy coat with Ficoll-Paque™ PLUS after centrifugation (schematic).

Material and Methods

Materials used

> SpinPro® 6 R centrifuge
> Rotor S-2×Universal ID with Universal Adapter 2
> Eppendorf Conical Tubes 50 mL
> Easypet® 3
> Eppendorf Serological Pipets 25 mL
> Eppendorf wide mouth flask 400 mL
> Ficoll-Paque™ PLUS, Cytiva
> Dulbecco's Phosphate Buffered Saline 1x (D-PBS), Gibco™
> Buffy Coats (BC) by Research Donors, Biomol GmbH

Recommended Standard Method

Experiment preparation

> Bring Ficoll-Paque™ Plus and DPBS to room temperature (18 – 21°C).
> Invert Ficoll-Paque™ Plus several times to mix.
> Wipe blood pouch with 70 % ethanol.

Sample processing

> Cut lower tube of blood pouch and transfer the blood to a wide-mouth flask in a sterile manner.
> Dilute the blood 1:2 in DPBS, close the bottle and mix gently.
> Pre-layer 15 mL of Ficoll-Paque™ PLUS into each of the 50 mL Eppendorf Conical Tubes.
> Overlay the Ficoll-Paque carefully with the blood/DPBS mixture using a serological pipette at the lowest speed setting.

Centrifugation

> Centrifuge samples with following parameters:
  > Time: 30 min, Time count setting: immediately
  > Temperature: 21°C
  > Speed: 400 rcf
  > Ramp settings: Acceleration 1, Deceleration 0
> Carefully remove the tubes to avoid mixing phases.

Evaluation of results

The quality of separation was determined by visual control. A defined interphase with clear phase transitions indicates optimal separation and high purity, whereas turbidity of the liquid implies suboptimal separation of PBMC.

Results and Discussion

In this Short Protocol the SpinPro® 6 R centrifuge was used for the isolation of PBMC from buffy coats using Ficoll-Paque PLUS.
The visual inspection of the samples after centrifugation revealed a very clear interphase between plasma and Ficoll-Paque (Fig. 3), which is widely regarded as a strong indicator of successful separation [4] and suggests a high quality and yield of PBMC. The overall run time for the protocol is approximately 43 minutes, with reduced acceleration and deceleration settings accounting for 13 minutes of this total time. A clear and distinct interphase was obtained with both, a load of 18 samples (Fig. 3a, fully loaded rotor) and a load of two (2) samples (Fig. 3b) demonstrating that the SpinPro 6 R centrifuge enables reliable and reproducible separation of PBMC Ficoll density gradient centrifugation.

Moreover, the results indicate that the Rotor S-2×Universal ID enables centrifugation with low vibrations, which is a critical requirement for all gradient centrifugation applications. This is especially important for PBMC isolation, as even minor vibrations can disrupt the interphase and compromise both purity and recovery of the separated cells.
The experiments were done in 50 mL conical tubes and can be adapted for smaller sample volumes. Strict adherence to standardized conditions ensures a high-yield and purity as well as viability of the isolated cells and enables reproducibility of the results.

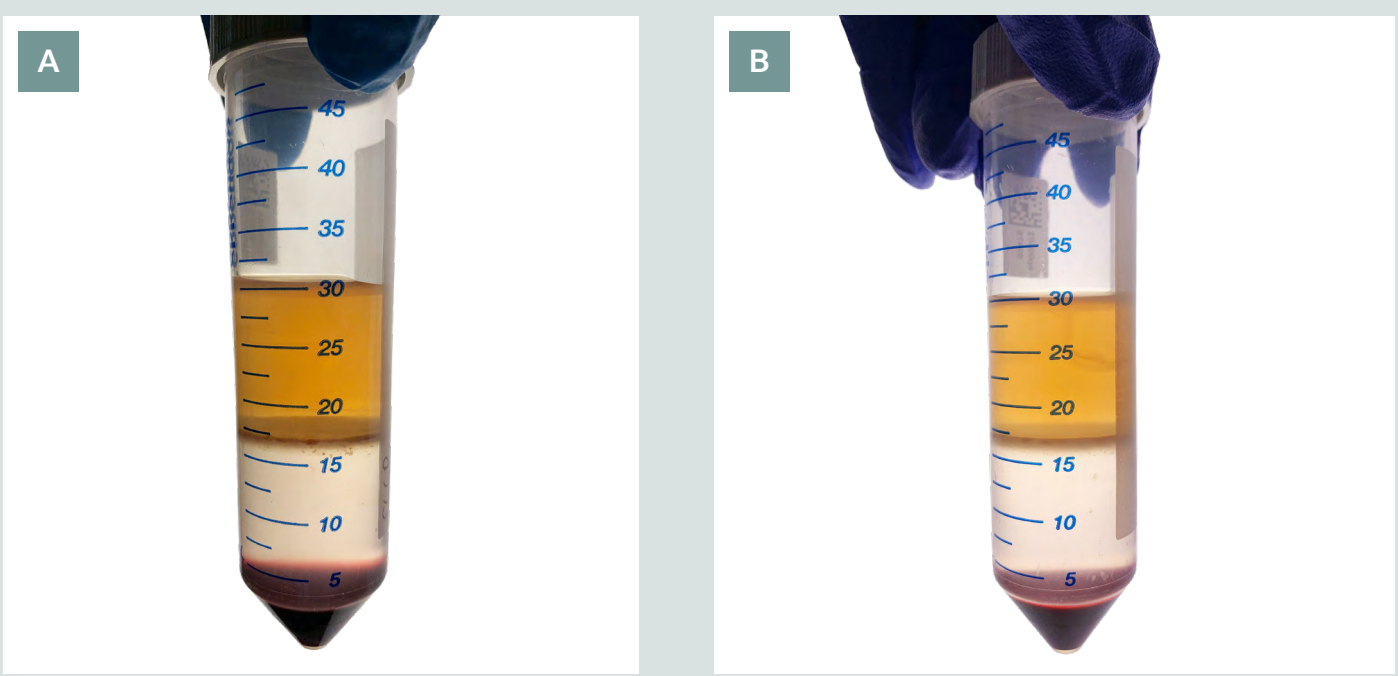

Figure 3: Results obtained after density gradient centrifugation in the SpinPro® 6 R with Rotor S-2×Universal ID loaded with A) 18 samples and B) with 2 samples.

Literature

[1] Ficoll-Paque™ PLUS Instructions for Use: https://cdn.cytivalifesciences.com/api/public/content/digi-12637-pdf
[2] Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. Scan. J. Clin. Lab. Invest. 1968, 21 (Suppl. 97): 77-89.
[3] Bøyum A. Isolation of lymphocytes, granulocytes and macrophages. Scan. J. Immunology. 1976, 5 (Suppl. 5): 9-15.
[4] Patrone D. et al. Optimization of peripheral blood mononuclear cell extraction from small volume of blood samples: Potential implications for children-related diseases. Methods Protoc. 2022, 5: 20.

Ordering information

Description	Order no.
SpinPro® 6 R with Rotor S-2xUniversal ID	See QR code below table
Universal adapter 2 for microplates and 25/50 mL conical tubes	5910769008
Caps for Rotor S-4xUniversal, aerosol-tight	5910750005
Easypet® 3 1-channel, 0.1 – 100 mL, incl. mains/power supply device, wall mounting device, shelf stand, 2 membrane filters 0.45 µm	4430000018

For ordering information, please check our local website or contact your local sales representative.
www.eppendorf.link/spinpro6r-centrifuge

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