

Separation of PBMCs from Blood Samples Using the New Thermo Scientific Benchtop 1-Liter Centrifuge

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Introduction

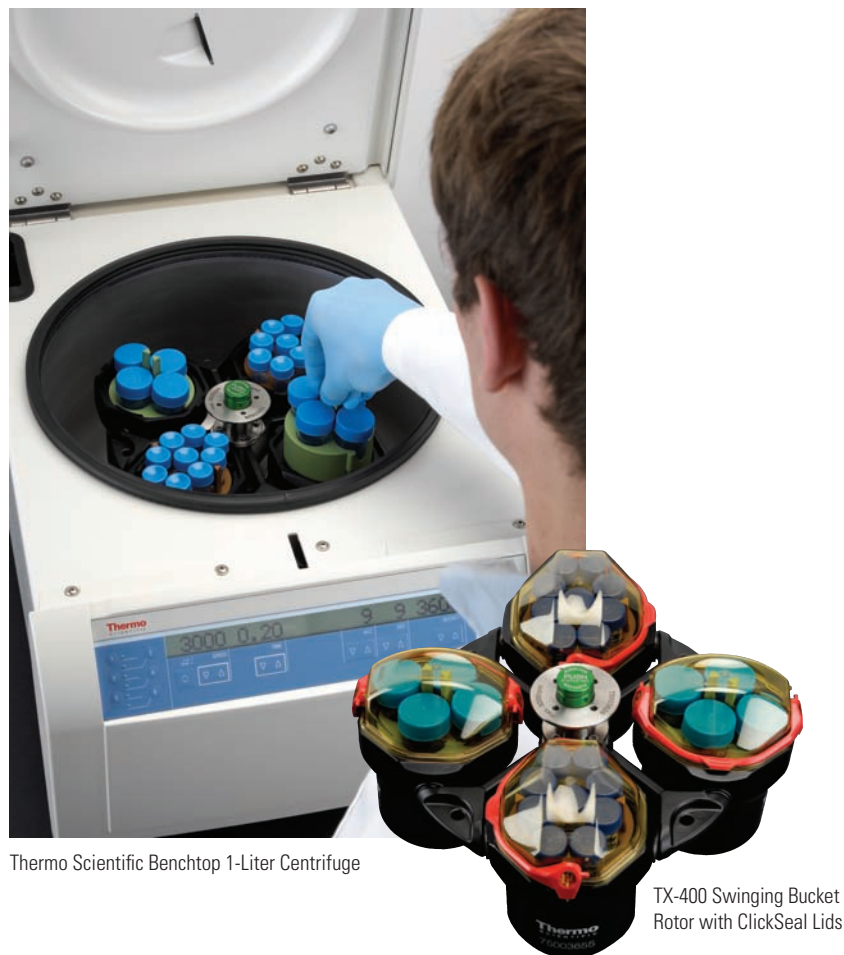
Peripheral Blood Mononuclear Cells (PBMCs) are blood cells with a round shaped nucleus, such as monocytes and lymphocytes, with the lymphocyte population comprised of T cells, B cells and NK cells. These cells are a critical component of the immune system, playing an integral role in the body's defenses.

Separation of PBMCs from whole blood is most commonly accomplished through density gradient centrifugation using Ficoll¹⁻⁵. After the centrifugation step, Ficoll separates layers of blood, with lymphocytes and monocytes under a layer of plasma. PBMCs are widely used in both research and clinical laboratories and the separation of PBMCs from blood by centrifugation constitutes an extremely important step for all subsequent analyses, such as in immune monitoring. An ineffective isolation of the cells can significantly affect immune monitoring, leading to unreliable results, particularly for antigen-specific T cells with lower frequency in the circulation⁶.

Two PBMC preparation methods are described in this application brief: Ficoll density gradient separation (Ficoll) and Cell Preparation Tubes (CPT), both using the new 1-liter Thermo Scientific general purpose centrifuge with a swinging bucket rotor. The sample preparation procedures were evaluated by assessing the cellular viability and cellular recovery.

PROTOCOL 1: Isolation of PBMC by Ficoll density gradient separation

The Ficoll density gradient separation of whole blood remains the most commonly used procedure for separation of mononuclear cells. This method, as described below, is a labor-intensive process requiring an operator with great technical expertise.



Thermo Scientific Benchtop 1-Liter Centrifuge

TX-400 Swinging Bucket Rotor with ClickSeal Lids

1. Collect blood into blood collection tubes.
2. In the laminar flow cabinet Class II, transfer blood from each blood collection tube into a 50 mL tube.
3. Dilute blood with PBS (1:1 dilution).
4. Carefully layer diluted whole blood over a Ficoll medium; the diluted blood is added to the gradient by gently pipetting onto the separation medium with the tubes held at an angle. This method requires considerable practice.
5. Spin at 833 xg (2125 rpm with the new 1-liter Thermo Scientific general purpose centrifuge) for 20 min, at 20°C, acceleration 9, no brake (braking rate 0).

Recommendation: Use the Thermo Scientific TX-400 swinging bucket rotor with ClickSeal® biocontainment lids to prevent the release of hazardous aerosols and protect from spills during centrifugation (see Table 1). These sealed buckets are certified for biocontainment by the Centre for Applied Microbiology and Research at Porton Down, U.K.

Note: To obtain good separations, it is critical that a clear separation be kept between the dense Ficoll medium and the blood layer before centrifugation.

6. Carefully remove the tubes from the centrifuge while not disturbing the layering.

KEY WORDS

- PBMCs
- Whole Blood
- Ficoll
- Cell Preparation Tubes (CPT)
- 1-Liter Benchtop Centrifuge

7. Carefully remove the PBMC layer from the tube and transfer to a new 15 mL (or 50 mL) conical tube. Avoid aspirating Ficoll.
 8. Discard the remainder of the Ficoll and red blood cells in closed tubes.
 9. Wash PBMC by adding enough PBS to make up 15 mL (or 50 mL).
 10. Spin at 425 xg (1518 rpm with the new Thermo Scientific 1-liter general purpose centrifuge) for 10 min, acceleration 9, deceleration 9.
 11. Decant the supernatant, loosen pellet and wash once again in PBS.
 12. Decant supernatant, loosen pellet and resuspend the cells in the appropriate volume of PBS for subsequent assay or procedure.
 13. Count the cells with the hemocytometer and determine the cell viability with trypan blue* by mixing a small volume (10 μ L) of the PBMC with trypan blue solution 1:1 in a microtiter plate. Load the hemocytometer with the cell mixture and wait for at least 30 seconds before counting.
4. After centrifugation, carefully open the CPT into a biological safety cabinet II. Using a Pasteur pipette, gently collect the mononuclear cells, which can be found in the layer just under the plasma.
 5. Transfer cells to a 15 mL (or 50 mL) conical tube. Avoid vigorous pipetting that would disintegrate the gel plug itself.
 6. Add PBS to wash cells. Mix cells by inverting tube 3 to 5 times.
 7. Centrifuge at 300 xg (approximately 1275 rpm with the new Thermo Scientific 1-liter general purpose centrifuge) for 15 min. Discard supernatant without disturbing cell pellet.
 8. Resuspend cell pellet by gently tapping tube with index finger.
 9. Add PBS and mix cells by inverting tube 3 to 5 times.
 10. Centrifuge at 300 xg for 10 min. Discard supernatant without disturbing cell pellet.

*The Trypan Blue Exclusion Test of Cell Viability is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, whereas dead cells do not.

PROTOCOL 2: Isolation of PBMC using Cell Preparation Tubes

The BD Vacutainer® CPT™ Cell Preparation Tube with Sodium Citrate (CPT) is a single tube system for the collection of whole blood and the separation of mononuclear cells. Isolation of PBMC in these tubes occurred according to the manufacturer's instructions:

1. Collect blood into CPT using venipuncture technique.
- Note: Blood tubes should be centrifuged within 2 hours of blood collection for best results.
2. Remix the blood sample immediately prior to centrifugation by gently inverting the tube 8 to 10 times.
 3. Centrifuge CPT tubes at 1700 xg (approx. 3037 rpm with the new Thermo Scientific 1-liter general purpose centrifuge) for 20 min at room temperature, acceleration 9 and deceleration 9.

Note: Do not centrifuge CPT over 2000 xg, as it may cause tube breakage.

Result

After centrifugation, the blood sample is separated showing layers from top to bottom:

- CPT: Plasma/platelets, PBMC, density gradient liquid, separation gel and red blood cells/granulocytes (Figure 1(a)).
- Ficoll density gradient: Plasma/platelets, PBMC, Ficoll and red blood cells/granulocytes (Figure 1(b)).

The sample preparation procedure carried out with Ficoll density gradient separation and with CPT was evaluated by assessing cellular viability and cellular recovery.

Cellular Viability

The viability (percent of live cells) of the fresh PBMC was assessed immediately after PBMC preparation. The viability of Ficoll-processed fresh PBMC and CPT-processed fresh PBMC were greater than 98% for both processing methods.

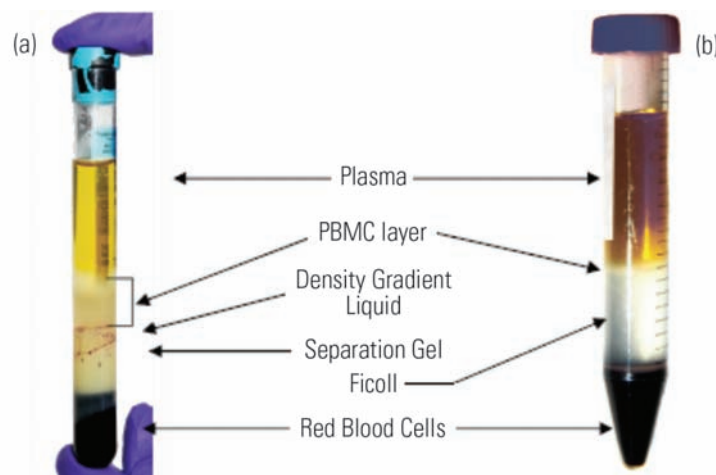


Figure 1: Typical PBMC layers after centrifugation in BD Vacutainer CPT (a) and Ficoll density gradient (b). The separation was performed with the Thermo Scientific 1-liter general purpose centrifuge.

11. Resuspend cell pellet in the appropriate volume of PBS for subsequent assay or procedure.
12. Count the cells with the hemocytometer and determine the cell viability with trypan blue (see step 13 in Protocol 1).

Note: These protocols can be modified to also work with the Thermo Scientific TX-200 swinging bucket rotor.

Cellular Recovery

The mean number of viable cells recovered from CPT-processed fresh PBMC was 2.66×10^6 viable cells/mL of blood and the mean yield of Ficoll-processed fresh PBMC was 2.96×10^6 viable cells/mL of blood. There were no significant differences between the recoveries of fresh PBMC processed using Ficoll or CPT so both methods are efficient for PBMCs isolation.

Conclusion

This application brief demonstrates that the new 1-liter Thermo Scientific general purpose centrifuge with the TX-400 swinging bucket rotor enables reliable and reproducible preparation of PBMCs by Ficoll density gradient and CPT in an environmentally safe manner by using ClickSeal biocontainment lids. PBMCs were processed and as a result, enough viable cells were available after PBMC isolation, that were able to respond to activation stimuli. Excellent cellular viability (>98%) and high yield cells were obtained for all subsequent analyses.

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Table 1: Accessories for the Thermo Scientific TX-400 Swinging Bucket Rotor (cat. no. 75003629)

DESCRIPTION	CAT. NO.
Round Buckets (set of 4)	75003655
ClickSeal Biocontainment Lids for Round Buckets (set of 4)	75003656
Adapters for 50 mL Conical Tubes (capacity of 16x50 mL)	75003683
Adapters for 15 mL Conical Tubes (capacity of 36x15 mL)	75003682
Adapters for 10 mL Blood Tubes (capacity of 56x10 mL)	75003681

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