

ISOLATE MONONUCLEAR CELLS DIRECTLY FROM WHOLE BLOOD

With EasySep™ Direct



Obtaining peripheral blood mononuclear cells (PBMCs) from blood samples with traditional methods, such as density gradient centrifugation, can be time consuming and difficult to automate. In addition, isolating cells from older blood samples can result in red blood cell (RBC) and granulocyte contamination, requiring additional RBC lysis and centrifugations.

Using EasySep™ Direct Human PBMC Isolation Kit (Catalog #19654), you can immunomagnetically obtain PBMCs without lysis or centrifugation in as little as 20 minutes. The resulting cells are highly purified with no RBC contamination, even on older samples (Figure 1 and 2).

To meet all of your laboratory needs, the EasySep™ Direct Human PBMC Isolation Kit can be used on:

- Whole blood
- Cord blood
- Bone marrow
- Buffy coats
- Leukapheresis products
- Leukocyte reduction system (LRS) cones

Increase sample processing throughput by automating your PBMC isolation with RoboSep™ instruments. Automation can minimize sample handling and free up valuable hands-on time (Table 1, page 3).

Why Use EasySep™ Direct Human PBMC Isolation Kit?

FAST. Isolate human PBMCs in as little as 20 minutes without the need for lysis or centrifugation.

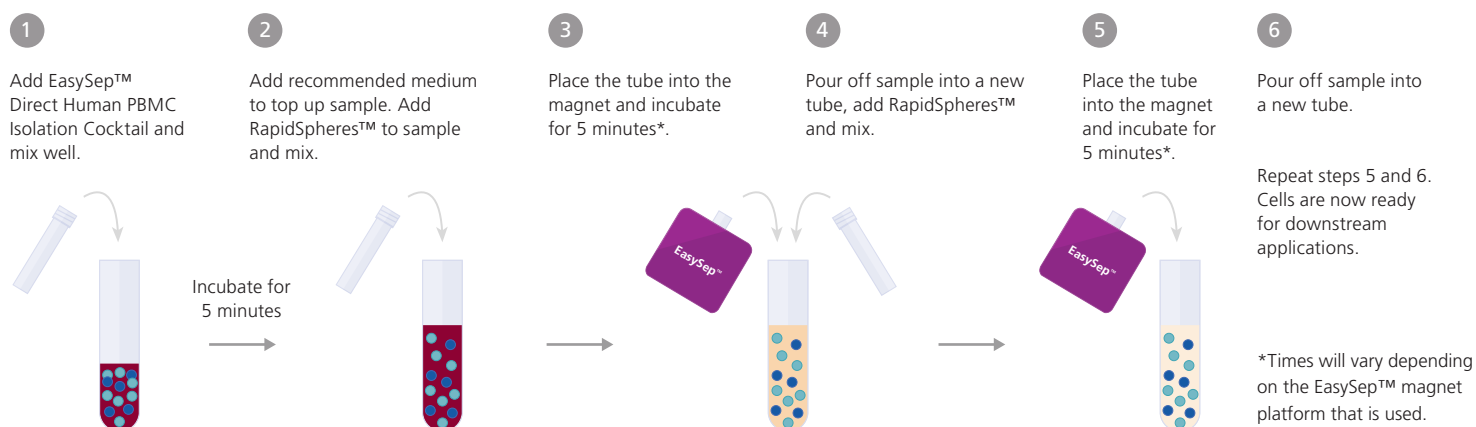
EASY. Avoid laborious and time consuming methods such as density gradient centrifugation and RBC lysis.

HIGH PURITY. Obtain highly purified mononuclear cells, even on older blood samples.

CONVENIENT. Automate blood sample processing with RoboSep™ instruments to increase laboratory throughput.

FLEXIBLE. Obtain cells from whole blood, cord blood, bone marrow, buffy coats, leukapheresis products, and leukocyte reduction system (LRS) cones.

How Does It Work?



Sample Protocol Video: www.stemcell.com/easysepdirectvideo

PBMC Isolation with EasySep™ Direct

Skip centrifugation and isolate PBMC immunomagnetically directly from whole blood with EasySep™ Direct Human PBMC Isolation Kit. With immunomagnetic cell isolation PBMCs are separated based on cell surface markers rather than cell density, resulting in highly purified PBMCs and fewer contaminating cells, even when processing older blood samples (Figure 2C).

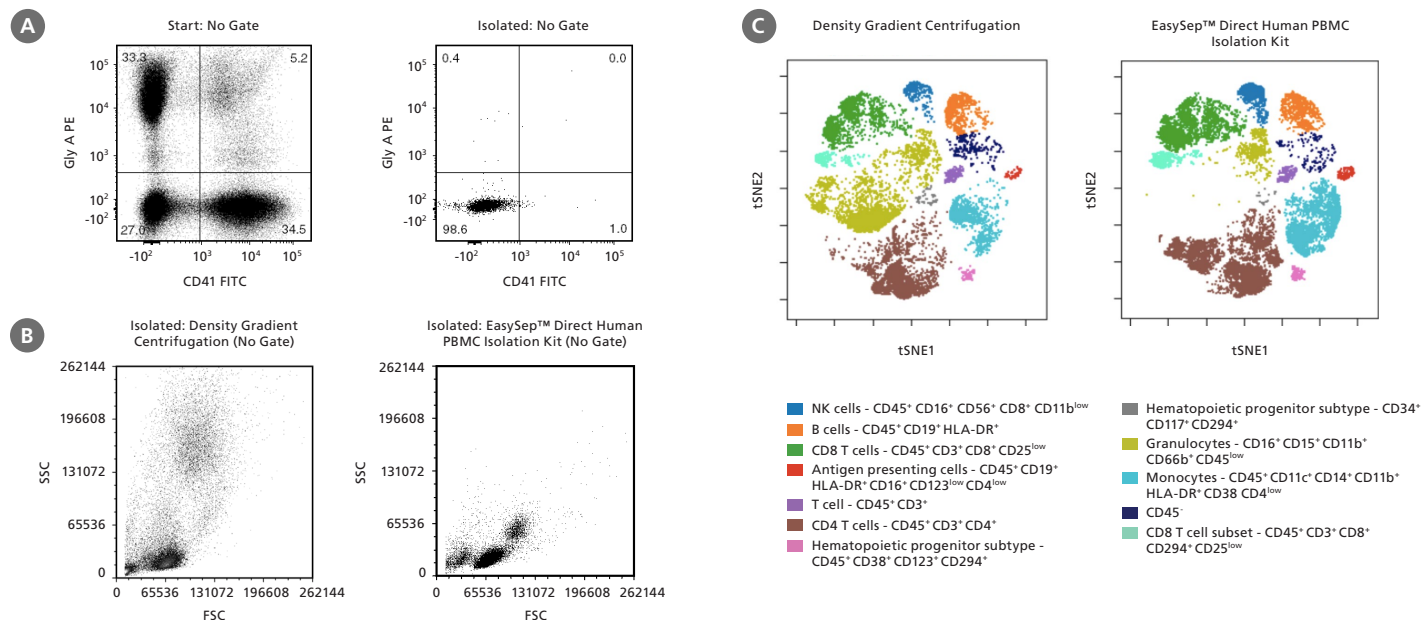


Figure 1. Typical EasySep™ Direct Human PBMC Isolation Profile

(A) Starting with human whole blood from normal healthy donors, the typical mononuclear cell content of the non-lysed final isolated fraction is $98.3 \pm 2.8\%$ (gated on CD45). In the above example, the mononuclear cell content of the whole blood start sample (lysed by ammonium chloride) and non-lysed final isolated fraction is 27.0% and 98.6% (not gated on CD45), respectively. (B and C) Mononuclear cells were isolated from whole blood samples using either density gradient centrifugation or EasySep™ Direct Human PBMC Isolation Kit. (B) Representative FSC vs. SSC flow cytometry plots. (C) Representative t-SNE plots of PBMCs stained with 19 markers and analyzed with mass cytometry (CyTOF). Cells are clustered and colored based on the combination of markers they express. Both density gradient centrifugation and EasySep™ Direct Human PBMC Isolation Kit resulted in comparable cell populations with the exception of the contaminating granulocyte population ($CD16^+ CD15^+ CD11b^+ CD66b^+ CD45^{low}$).

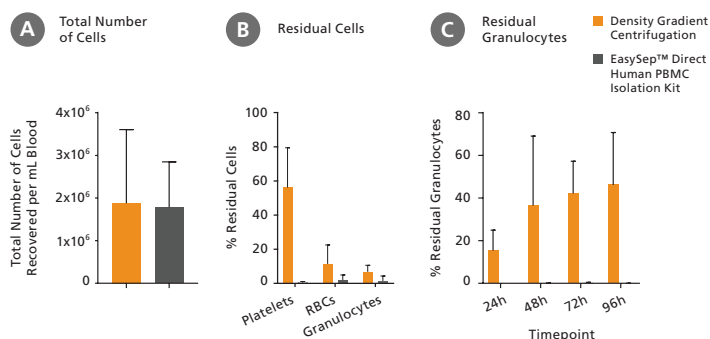


Figure 2. EasySep™ Direct Human PBMC Isolation Kit Results in Fewer Contaminating Cells Compared to Density Gradient Centrifugation

Mononuclear cells were isolated from whole blood samples using either density gradient centrifugation or EasySep™ Direct Human PBMC Isolation Kit. Cells were counted and analyzed by flow cytometry. (A) Both density gradient centrifugation and EasySep™ Direct Human PBMC Isolation Kit resulted in an equivalent total number of nucleated cells recovered from 24-hour-old blood samples (mean \pm SD; $n=14$). (B) Using EasySep™ Direct Human PBMC Isolation Kit to obtain mononuclear cells from 24-hour-blood samples resulted in lower numbers of residual platelets ($CD41^+$), red blood cells (Glycophorin A $^+$ /CD45 $^-$), and granulocytes ($CD66b^+$) compared to density gradient centrifugation (mean \pm SD; $n=15$). (C) Cell isolation from 24-, 48-, 72-, and 96-hour-old blood samples using EasySep™ Direct Human PBMC Isolation Kit resulted in lower numbers of residual granulocytes compared to cell isolation using density gradient centrifugation (mean \pm SD; $n=3$).

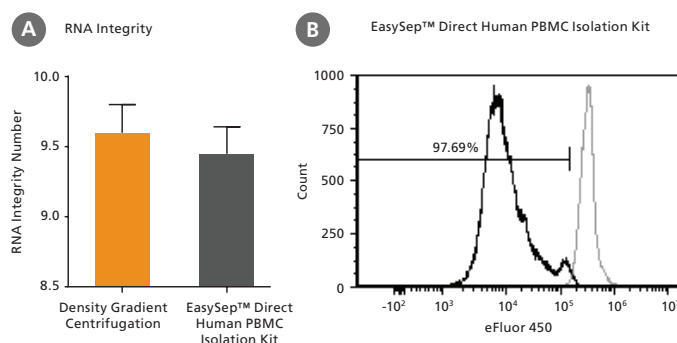


Figure 3. PBMCs Isolated with EasySep™ Direct Human PBMC Isolation Kit Proliferate and Maintain High RNA Integrity

Mononuclear cells were isolated from whole blood samples using either density gradient centrifugation or EasySep™ Direct Human PBMC Isolation Kit. (A) Isolated mononuclear cells were used for downstream RNA isolation. There was no significant difference in RNA integrity as measured with the Agilent RNA Bioanalyzer (mean \pm SEM, $n=3$). (B) Isolated mononuclear cells were labeled with Proliferation Dye eFluor 450 and stimulated with ImmunoCult™ Human CD3/CD28 T Cell Activator and 0.5 ng/ml IL-2. After 4 days in culture, cells were analyzed for proliferation by flow cytometry. Representative histogram showing dividing cells (eFluor 450 low).

Automating PBMC Isolation with RoboSep™

Minimize sample handling and free up valuable hands-on time by automating PBMC isolation with the EasySep™ Direct Human PBMC Isolation Kit and the RoboSep™-S instrument. By automating all sample labeling and magnetic separation steps you can perform simultaneous cell isolations from up to 4 samples and increase sample throughput.

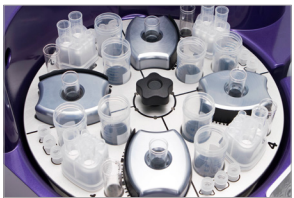
How Does It Work?



1 Select desired protocol and load samples, EasySep™ reagents, buffer, and tips.



2 Press "Run".



3 Return in 30 minutes to collect your separated cells.

Table 1. Automated PBMC Isolation Using RoboSep™-S Reduces the Overall Cell Separation Time Compared to Density Gradient Centrifugation

Step	Duration (minutes)		
	Manual Density Gradient Centrifugation	Manual EasySep™ Direct	Automated EasySep™ Direct on RoboSep™-S
Preparation of tubes with density gradient medium and layering sample	7	-	-
Loading samples and reagents onto the RoboSep™-S instrument	-	-	5
Cell isolation	45	20	30
Cell collection	10	1	1
Optional: Wash step to remove platelets	15	-	-
Optional: Red blood cell lysis	15	-	-
Final centrifugation step	15	Optional	Optional
Total time for cell separation	≥ 77 Minutes	~ 21 Minutes	~ 36 Minutes
Total hands-on time	≥ 32 Minutes	~ 21 Minutes	~ 6 Minutes

Steps and approximate times to isolate mononuclear cells from whole blood samples when following using density gradient centrifugation or EasySep™ Direct Human PBMC Isolation Kit (manual separation or automated on the RoboSep™-S). For density gradient centrifugation, additional washing steps and red blood cell lysis might be required.

Why Automate PBMC Isolation with RoboSep™?

- EFFICIENT.** Minimize hands-on time and increase sample throughput.
- HIGH PURITY.** Obtain highly purified mononuclear cells with improved RBC, granulocyte, and platelet clearance compared to density gradient centrifugation.
- CONVENIENT.** Load your samples and reagents, press "Run", and come back to your isolated cells.
- FLEXIBLE.** Obtain cells from cord blood, bone marrow, buffy coats, leukapheresis products, and leukocyte reduction system (LRS) cones.

Special Application

Automate PBMC Isolation from a Full-Size Leukapheresis Pack

Obtain PBMCs from a full-size leukapheresis pack in a single run with EasySep™ Direct Human PBMC Isolation Kit and RoboSep™-S. This automated cell isolation system results in fewer contaminating cells compared to density gradient centrifugation (Figure 4). First, concentrate the leukapheresis pack (see below). Then, load the tubes with the resuspended cells, as well as the EasySep™ reagents, buffers, and tips onto the instrument. Start the instrument's isolation protocol and come back in 30 minutes to collect your isolated cells.

Below is an example of how to concentrate an entire leukapheresis pack for downstream PBMC isolation using RoboSep™-S. For more information please consult the product information sheet for your specific product or contact our Product & Scientific Support team at techsupport@stemcell.com.

1. Transfer the entire contents of the leukapheresis bag into tubes and centrifuge at 300 x g for 10 minutes with the brake on.
2. Carefully remove the supernatant and resuspend the cells in EasySep™ Buffer (Catalog #20144) to obtain a final volume of 20 mL and approximate concentration of 4×10^8 cells/mL.
3. Add EDTA at 6 mM final concentration and aliquot the 20 mL into four 14 mL polystyrene round-bottom tubes (e.g. Catalog #38008). Each tube will now contain 5 mL of resuspended cells.
4. Select the appropriate protocol on the screen.
5. Follow the onscreen prompts and load the 4 x 14 mL tubes, reagents, tips and buffer onto the RoboSep™-S carousel.
6. Press "Run".
7. Come back in 30 minutes after the run is complete and collect the isolated cells.



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*Certain products only available in select territories.

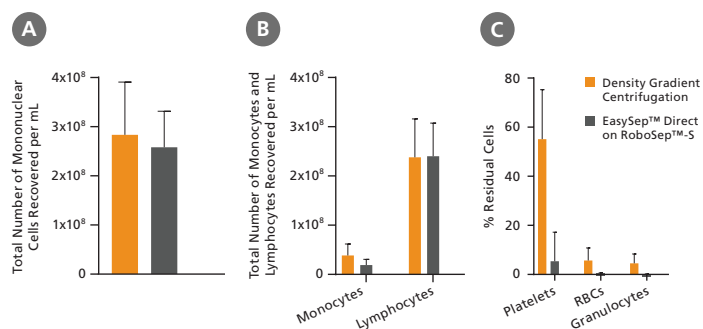


Figure 4. PBMC Isolation from a Full-Size Leukapheresis Pack Using the EasySep™ Direct Human PBMC Isolation Kit Automated with RoboSep™-S Results in Fewer Contaminating Cells Compared to Density Gradient Centrifugation

Mononuclear cells were isolated from single concentrated leukapheresis packs using either density gradient centrifugation with Lymphoprep™ density gradient medium (Density Gradient Centrifugation) or EasySep™ Direct Human PBMC Isolation Kit automated on the RoboSep™-S instrument (EasySep™ Direct on RoboSep™-S). Cells were counted and analyzed by flow cytometry. Compared to density gradient centrifugation, EasySep™ Direct Human PBMC Isolation Kit automated on the RoboSep™-S instrument resulted in (A) equivalent numbers of total mononuclear cells, (B) equivalent numbers of total monocytes and lymphocytes and (C) lower numbers of residual platelets (CD41⁺), red blood cells (Glycophorin A⁺ /CD45⁻), and granulocytes (CD66b⁺) (mean ± SD n=6).

Product	Blood Volume Processed	Catalog #
EasySep™ Direct Human PBMC Isolation Kit	100 mL	19654
EasySep™ Direct Human PBMC Isolation Kit for RoboSep™	100 mL	19654RF

RoboSep™ Instruments for Automated Cell Isolation

RoboSep™-S and RoboSep™-16 fully automate all cell labeling and separation steps of the EasySep™ procedure, minimizing sample handling and freeing up technician time. Setup is simple: just load your samples and reagents and walk away. Return later to separated cells, which are immediately ready for downstream applications.



RoboSep™-S
Catalog #21000



RoboSep™-16
Catalog #23000

To see RoboSep™ instruments in action, visit www.RoboSep.com.

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