



## Application Note

# Performance Characterization of the Purecell™ Select System for Enrichment of Mononuclear Cells from Human Whole Blood

### Introduction

Enriched mononuclear cells (MNC) from whole blood (WB) are routinely used in numerous research applications. Centrifugation-based methods utilizing density gradient reagents are regularly performed for MNC enrichment and/or red blood cell (RBC) debulking. While there are many density media commercially available, Ficoll\* is the gold standard for this basic cell processing. Ficoll processing occurs in an open system, with multiple labor- and time-intensive steps, many of which contribute to overall less-than-desired recovery and processing reproducibility. There is a clear need for a cell processing system that overcomes the disadvantages of density gradient-based methods. A simple, robust processing method with improved cell recoveries and faster processing times is necessary.

Pall has directed its extensive knowledge and experience in blood cell interaction with fibrous materials and medical device development toward addressing these processing challenges. The result is the introduction of the Purecell Select System, utilizing Pall's specialty materials, device, and protocol. The Purecell Select System eliminates open-tube processing and is designed for high MNC recovery from a rapid (8-15 minutes), highly reproducible and easy-to-use system. Additionally, the Purecell Select System has a sterile fluid path with very few points of direct user interaction.

The process is simple. First, the sample is transferred to the sample bag via a sterile Luer-Lok\* port. The sample flows through the system under gravity flow, and MNC are captured within the filter. After flow has stopped, the user back-flushes the filter with the optimized Harvest Solution to recover the enriched MNC population. In this Application Note, performance characterization of the Purecell Select System is provided as a guide for customers. In these studies, typical MNC and total white blood cell (WBC) recovery, viability and system

reproducibility using WB is provided. In addition, results from WB collected into different anticoagulants and fresh versus 24-hour WB is reported. Data from WB volumes of 20-120 mL is also provided. The Purecell Select System is tolerant of these variables, showing high recoveries and good viability of MNC in all cases.

The Purecell Select System's speed and ease of use hold great potential to advance the technology for enriching rare cells or abundant MNC for routine use in cell-based studies in the research environment. With minimal sample manipulation during processing, there is a significant reduction in the risk of contamination and user-to-user variation. Elimination of open-tube steps and use of a needleless system reduce the risk of contamination and user exposure to bio-hazard material.

### Materials and Methods

#### Human WB Samples

- For most experiments, fresh units of human whole blood collected in CPDA-1 anticoagulant in a blood bag are held at ambient temperature until use, unless otherwise noted in figure legend and results section.
- Experiments are performed within 3 hours of blood collection, except for experiments using 24-hour WB, as noted in figure legend.

#### Mononuclear Cell Enrichment by Filtration (8-15 minutes total processing time)

- A syringe is used to transfer WB from blood bag to filtration set. 50 mL is used for all experiments unless otherwise noted.
- The WB passes through the Purecell Select System by gravity flow.
- Cells are recovered by back-flushing (i.e., reversing the direction of the flow) with 24 mL sterile Purecell Select System Harvest Solution.

## Materials and Methods *(continued)*

### Mononuclear Cell Enrichment Using Ficoll-Paque\*

- 2 or 8 mL of WB is diluted with an equal volume of phosphate buffer saline (PBS) and carefully layered onto 3 or 10 mL Ficoll-Paque (GE Healthcare) in a sterile 15 or 50 mL conical centrifugation tube (Falcon).
- The tubes are centrifuged at 400 x g for 30 minutes at room temperature.
- The interface layer containing the mononuclear cells is removed and transferred to a clean tube, and washed twice with 15 mL of sterile PBS.
- The cells are centrifuged at 700 x g for 10 minutes.
- The final cell pellet is resuspended in PBS or Iscove's Media with 2% FBS.

**Cell counts** are generated on a Cell-Dyn\* 1800 hematology analyzer (Abbott Labs) following standard protocols. Triplicate measurements are averaged for the concentrations of WBC, RBC, granulocytes, mids (monocytes and other similarly-sized cells), lymphocytes and platelets. Percent recoveries for each sub-population are determined by calculating the number of cells before and after the preparation using the following formula:

$$\left( \frac{\text{Concentration of cells after isolation procedure x volume}}{\text{Concentration of cells before isolation procedure x WB volume}} \right) \times 100$$

Total number of MNC is determined by the following formula:

$$(\text{Concentration of lymphocyte + monocytes}) \times \text{volume}$$

Coefficient of Variation (CV) is determined by the following formula:

$$(\text{Standard deviation} / \text{mean}) \times 100$$

**Three part differential** for some experiments is based on WBC counts from Cell-Dyn and lymphocyte, monocyte and granulocyte percentages from forward versus side scatter plots on a BD FACSCalibur\* flow cytometer (BD Biosciences). In other cases, these population determinations are based on phenotypic data. The formula used is as follows:

$$(\text{Concentration of WBC x volume x percent of population}) / 100$$

**Phenotype analysis** by flow cytometry for cellular composition of WB and cell mixtures obtained with the Purecell Select System or Ficoll processing methods. Cell surface marker antibody staining followed by analysis with the BD FACSCalibur flow cytometer is performed using standard protocols.

- Aliquots of cells are incubated with fluorophore-conjugated-antibodies to the differentiation markers CD3, CD14, CD19, CD16+CD56, CD66b and CD45 (BD Biosciences) for T cells, monocytes, B cells, NK cells, granulocytes, and leukocytes, respectively.
- RBC are lysed with 1 x H-lyse Buffer (R&D Systems).
- WBC are pelleted by centrifugation at 500 x g for 5 minutes, the supernatant is removed, and the cells are resuspended in PBS.

The percent positive for each of the surface markers in the WBC population is determined. The WBC count from the Cell-Dyn is used to calculate the total number of cells in each group using the following formula:

$$\frac{(\text{Concentration of WBC x volume x percent positive})}{100} = \text{number of cells in each group}$$

## Results

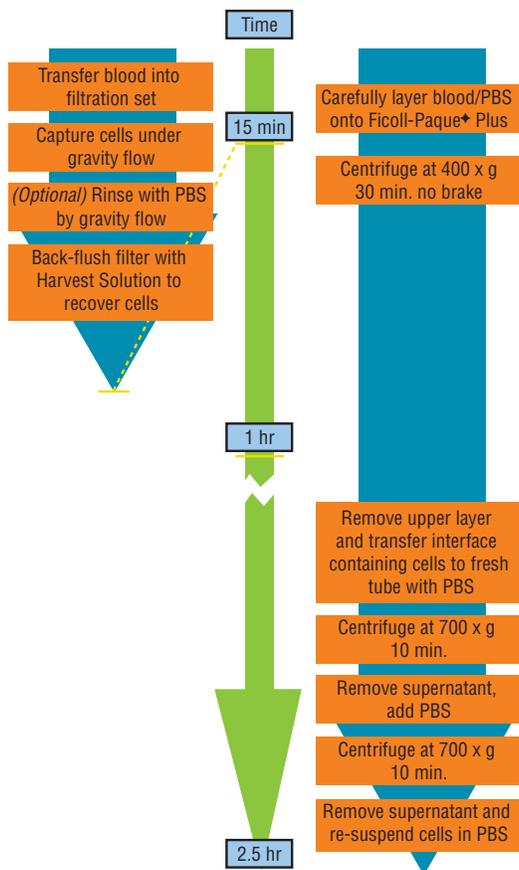
### Protocol Comparison

Although there are numerous ways to process WB for MNC enrichment, we have focused on the most widely used method – Ficoll density gradient centrifugation. The schematic in Figure 1 illustrates the major differences between these two methods (processing time and number of steps that directly involve the user). The Purecell Select System requires approximately 8-15 minutes of total processing time for 50 mL of WB, varying somewhat with changes in WB volume. In contrast, Ficoll processing can take 2.5 hours or more, depending on sample volume and number of samples processed. The Purecell Select System process has only three steps for user activity: transfer the sample to the input bag; back-flush the filter with Harvest Solution; and, remove the bag containing MNC enriched sample from the set. Capture of MNC on the Purecell Select System filter occurs as the sample passes through the filter under gravity flow. Conversely, Ficoll processing is labor intensive, requiring many more user steps, including two-fold sample dilution, careful layering of sample onto Ficoll, collection of cells at the Ficoll interface, multiple wash steps, and final resuspension of the cell pellet. The Purecell Select System, with fewer steps and a sterile fluid path, reduces risk of sample contamination as a result of processing. For users, the needleless system provides an added safety measure by minimizing risk of exposure to biohazard material. Additionally, minimal user involvement means that multiple samples can be processed at the same time. The system itself is very simple to use, and different users within the same laboratory will see equivalent performance.

## Results (continued)

**Figure 1**

Comparison of Experimental Procedures

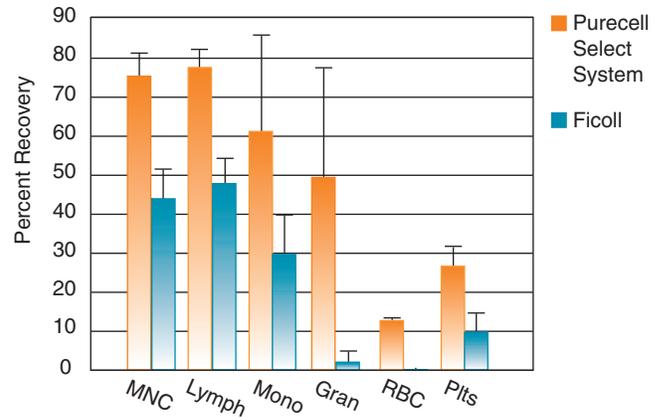


### Percent Recovery of the Major Cell Populations

Purecell Select System processing consistently results in higher recovery of the lymphocytes and monocytes as compared to Ficoll (orange bars, typically 65-95% and 45-90% for Purecell Select System lymphocytes and monocytes, respectively; Figure 2 and data not shown). MNC recovery from Ficoll processing of WB is significantly lower (blue bars, typically 25-60%; Figure 2 and data not shown). For all matched donor samples tested, percent MNC recovery is consistently 20-50% higher for Purecell Select System processing as compared to Ficoll density gradient processing. Purecell Select System processing typically results in 85-88% reduction in RBC and 65-85% reduction in platelets. The residual RBC are a function of the residual blood in the Purecell Select System device. Thus, as more WB is processed, the percent reduction in RBC increases (data not shown). Ficoll density gradient preparation of human WB results in higher reduction of RBC and granulocytes with variable reduction in platelets. Granulocyte and platelet removal with Ficoll processing is dependent on how cells are collected at the interface after the first centrifugation step.

**Figure 2**

Percent Recovery of Major Cell Populations



Percent recovery of major cell types in 50 mL WB starting material, Purecell Select System processed samples and Ficoll samples. Percent recovery is calculated based on WBC, platelet and RBC concentration (Cell-Dyn 1800), and percent lymphocytes, monocytes and granulocytes is based on phenotype data (BD FACSCalibur system). Duplicates are averaged. Means +/- 1 SD from 6 donors are reported.

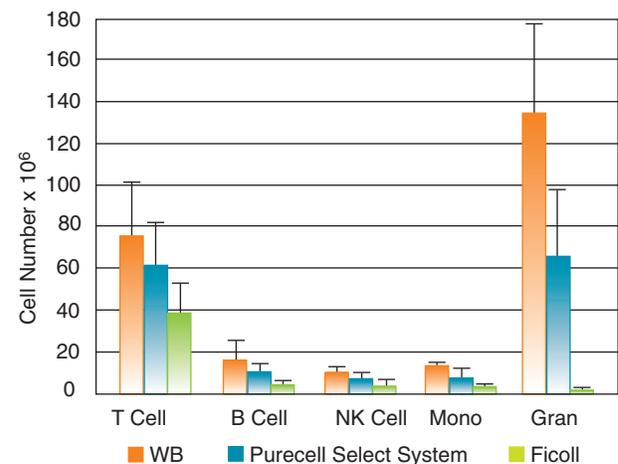
### Cellular Composition of the WBC Preparations and WB Starting Material

Phenotypic analysis of processed samples shows that the Purecell Select System yields more cells of all types than are recovered with Ficoll density gradient preparation (Figure 3). The Purecell Select System exhibits a higher average number of recovered cells for each leukocyte sub-group. This clearly demonstrates a much greater yield of major MNC populations as compared to Ficoll processing.

There is a great deal of donor-to-donor variation in pre- and post-processing cell numbers; hence, the large error bars. However, the Purecell Select System processing consistently results in 20-50% greater recovery of MNC. Data for abundant cell types is presented here. Recovery of rare or other cell types is described in other Pall application notes.

**Figure 3**

Total Cell Number of Major Leukocyte Populations



Total number of major cell types in 50 mL WB and processed samples. Phenotype data (BD FACSCaliber system) averages from 6 donors +/- 1 SD are reported.

## Results (continued)

### Purecell Select System Reproducibility, Single Donor

Reproducibility data from a single donor reflects the variability in the processing without the confounding variability across donors. A single donor WB sample is split into eight 50 mL aliquots and processed using the Purecell Select System. In addition, eight 2 mL aliquots from the same donor are processed by Ficoll density gradient centrifugation. Table 1 shows the average percent recovery along with CV determination (percent standard deviation) for the major cell populations. CVs for all WBC populations and platelets are significantly lower with Purecell Select System processing as compared to Ficoll density gradient processing, indicating much less variability in the WB processing method. RBC reduction levels are also highly reproducible. This is not a surprise as the simplicity of the method serves to limit the variability typically seen with Ficoll processing. Note that the Ficoll processing CVs on RBC, granulocytes, and platelets are unusually high because the total number of these cells is quite low in the Ficoll-processed product.

**Table 1**

Single Donor Reproducibility of Purecell Select System Versus Ficoll Processing Methods

		Percent Recovery						
		WBC	MNC	Lymph	Mono	Gran	RBC	Plt
Purecell Select System	Avg	58.7	80.9	84.7	63.9	42.2	13.8	21.8
	CV	2.7	2.0	2.0	4.3	5.4	2.2	13.1
Ficoll	Avg	19.5	43.2	47.5	24.0	2.1	0.1	2.8
	CV	8.5	7.8	8.1	11.3	20.2	30.0	26.5

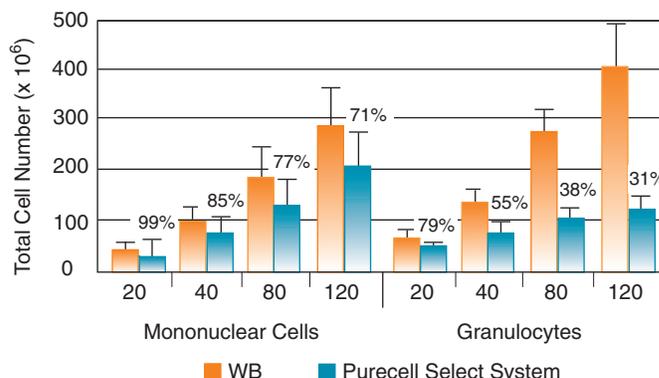
A single WB donor sample was split into 8 aliquots for processing with Purecell Select System and Ficoll density gradient. The average recovery is calculated from WBC number (Cell-Dyn 1800) and forward versus side scatter data from flow cytometer.

### WB Volume Testing, Purecell Select System Results Using 20-120 mL of WB

The bar graph in Figure 4 shows total number of MNC and granulocytes (left and right, respectively) and percent recovery from the Purecell Select System processing of 20, 40, 80, and 120 mL of human WB. As expected, the number of MNC and granulocytes in the Purecell Select System sample increases with increasing volume of WB (blue bars). There is a slight decrease in the percent recovery of MNC as the WB volume increases, as seen by the increasing gap between cell number in the WB sample (orange bars) and the Purecell Select System sample (blue bars) at higher volumes. Interestingly, there is a dramatic decrease in granulocyte recovery as the WB volume increases. This results in a relatively greater enrichment of MNC as compared to granulocytes with increasing volume. When the volume is increased to 150 mL, there is a significant reduction in percent MNC recovery (data not shown). Thus, 120 mL is the upper limit recommended for this filter harvest set design.

**Figure 4**

Purecell Select System Total Cell Number and Recoveries from 20-120 mL of WB



Total number and percentage of mononuclear cells and granulocytes in WB or Purecell Select System from indicated volume of WB (20, 40, 80, 120 mL). Samples processed in duplicate. 20 mL volume from 3 donors; all others from 5 separate donors.

### Comparison of Results From Fresh Versus Day-old WB

Data in Table 2 shows that fresh and 24-hour WB (from 6 donors) work equally well when processed using the Purecell Select System. There are no major differences in percent recovery of the MNC (Table 2) or cell composition of the samples (data not shown) between WB processed on the day of collection or approximately 24 hours after collection. In these experiments, processing of cells on the Purecell Select System showed no negative impact on the viability of cells. Post-processing viability was on average 4.2% (+/- 1.8) higher for fresh WB and 6% (+/- 6.1) higher for 24-hour WB samples when compared to the starting sample viability. This suggests that some of the non-viable cells are not recovered in the Purecell Select System sample.

**Table 2**

Fresh Versus 24-hour WB, Percent Recovery of MNC and Total WBC Population

Sample Type	MNC Recovery	WBC Recovery
Fresh WB - FH sample	80.1 +/- 9.9%	67.4 +/- 4.3%
24 hr. WB - FH sample	76.4 +/- 5.6%	68.8 +/- 5.7%

Comparison of MNC and total WBC recovery (Cell-Dyn data) from matched donors using fresh WB versus the same WB stored at 4 °C overnight. Chilled WB is equilibrated to room temperature prior to processing. Averages from 6 donors +/- 1 SD are reported.

## Results (continued)

### Comparison of Effect of Anticoagulants on Purecell Select System Cell Recovery, Major Cell Populations

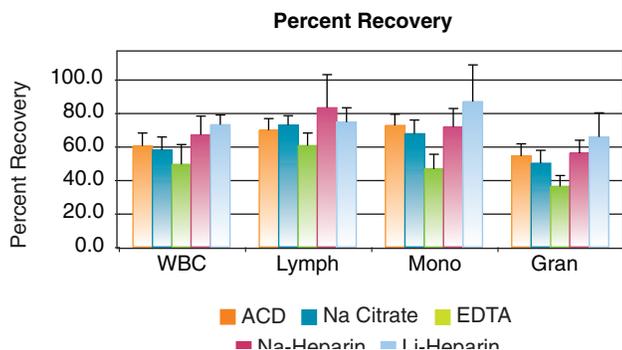
CPDA is the standard anticoagulant used for Purecell Select System experiments as it is known to be very friendly to blood cells. ACD and CPDA are commonly used anticoagulants in blood banks. Heparin and EDTA are typically used in research labs, with Na Citrate also used, but less frequently. An analysis of the impact of these anticoagulants on recovery of the major cell populations is shown in Figure 5. Single donor WB was collected into these 5 anticoagulants. The individual and averaged recovery data from 5 donors demonstrate that ACD and Na Citrate consistently have high WBC recovery and lower donor-to-donor variability (orange and blue bars). CPDA also gives excellent recoveries and reproducibility data not shown). Thus, ACD, CPDA, and Na Citrate anticoagulants are strongly recommended. There is evidence of reduced recovery of MNC and platelets from WB collected into EDTA (green bars). The effect of EDTA shows variability across donors, but monocyte and granulocyte recoveries are consistently the most effected. Li and Na Heparin gave the most variable results across donors and may adversely affect cell viability. Viability data from samples, measured at ~2 hours and ~22 hours post-processing, shows a slight negative trend on WBC viability with Heparin anticoagulant (data not shown). This effect is more apparent after ~24-hour storage at 4 °C for both WB and Purecell Select System samples. Although this effect is small, it has been reported by others.

## Conclusions

Customer discussions regarding characteristics most desired for MNC enrichment show three clear needs: fast sample processing, easy-to-use method, and high recovery. We show that Pall's Purecell Select System takes a significant step toward achieving these goals as well as reducing the risk of contamination by eliminating open-tube processing. The system is fast, processing 50 mL in ~8-15 minutes from start to finish. It is easy to use, requiring minimal training and hands-on time. The system provides a sterile fluid path and needleless ports for sample introduction and cell collection that are sterile and swabable. Additionally, high MNC recoveries (typically > 70% from 50 mL) are the norm, translating into 40-100% more MNC than Ficoll processing on matched donors. The system is sufficiently robust that good recovery and viability can be achieved using WB volumes of 20-120 mL, WB collected into any of the common anticoagulants, or day-old WB samples. The ability to recover over 200 million MNC in 10-20 minutes from a single 120 mL WB Purecell Select System run is appealing to most customers. Although we do not expect this system to replace Ficoll processing for all applications, it seems likely that these advantages will have significant benefits, particularly in cell-based research studies.

**Figure 5**

*Anticoagulant Effect on Recovery of Major Cell Populations*



The percentage recovery of WBC, lymphocytes, monocytes, and granulocytes in Purecell Select System samples using WB from single donors collected into 5 or 6 tubes (10 mL WB/tube) for each anticoagulant tested (ACD, EDTA, Na Citrate, Li-Heparin, Na-Heparin). Prior to processing, WB from tubes containing each anticoagulant is pooled. Recovery and composition are calculated based on cell concentration determination as measured on the Cell-Dyn 1800 hematology instrument and percent lymphocytes, monocytes and granulocytes based on forward and side scatter data from the BD FACSCalibur system. Error bars show SD across donors.



**Pall Medical - Cell Therapy**  
600 South Wagner Road  
Ann Arbor, MI 48103-9019 USA

800.521.1520 toll-free in USA  
734.665.0651 phone  
734.913.6495 fax  
Stem\_Cells@Pall.com email

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[www.pall.com/celltherapy](http://www.pall.com/celltherapy)

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**Australia** - Cheltenham, VIC  
Tel: 03 9584 8100  
1800 635 082 (in Australia)  
Fax: 1800 226 825

**Brazil** - Rio de Janeiro  
Tel: (5521) 2111.92.08  
Fax: (5521) 2111.92.05

**Canada** - Ontario  
Tel: 905.542.0330  
800.263.5910 (in Canada)  
Fax: 905.542.0331

**Canada** - Québec  
Tel: 514.332.7255  
800.435.6266 (in Canada)

**Germany** - Dreieich  
Tel: 06103.307 333  
Fax: 06103.307 399

**India** - Mumbai  
Tel: +91 22 67995555 or  
+91 22 66830700  
Fax: +91 22 67995556

**Korea** - Seoul  
Tel: 82.2.560.8711  
Fax: 82.2.569.9095

**Thailand** - Bangkok  
Tel: (66) 2937 1055  
Fax: (66) 2937 1066

**United Kingdom** - Portsmouth  
Tel: +44 (0)23 9230 3452  
Fax: +44 (0)23 9230 3324  
Biosvc@Pall.com

**United States** - California  
Tel: 626.339.7388  
Fax: 626.858.0458  
Stem\_Cells@Pall.com

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