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Akadeum Life Sciences, Inc.
674 S Wagner Rd
Ann Arbor, MI 48103

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Removal of Red Blood Cells from Human Peripheral Blood Mononuclear Cell Samples Using Akadeum Human Red Blood Cell Depletion Microbubbles

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734. 707. 1233
info@akadeum.com
www.akadeum.com

Removal of Red Blood Cells from Human Peripheral Blood Mononuclear Cell Samples Using Akadeum Human Red Blood Cell Depletion Microbubbles

Human peripheral blood mononuclear cells (PBMCs) are commonly used for studying immunological functions including cytokine production, proliferation, surface marker expression, and transcription factor expression. Typically, a density gradient technique, such as Ficoll®, is used to isolate mononuclear cells from peripheral blood or bone marrow, but these techniques may leave behind residual red blood cells (RBCs) in the final fraction of PBMCs due to disease state or poor preparation technique. A high percentage of RBC contamination can impede subsequent cell sorting or other single cell isolation instrumentation. For example, residual RBCs can extend sort times and occupy wells that are needed for analysis of the relevant cells. Ammonium Chloride Potassium (ACK) buffers can be added to selectively lyse the red blood cells, however, there are concerns about the viability of certain cell types in response to these types of buffers and the impact of additional cellular debris is not well characterized[1].

Akadeum Human Red Blood Cell Depletion Microbubbles

In this demonstration, PMBCs were obtained from whole blood and RBCs were re-introduced at a ratio of 1 to 10, resulting in 10% contamination (Figure 1). In order to remove the RBCs, Akadeum Human Red Blood Cell Depletion Microbubbles (13210-140) were added to the PBMC sample and the sample was mixed by triturating 30 times using a 200 µL pipet. After mixing, 1 mL of Separation Buffer (PBS containing 2mM EDTA and 0.5% bovine serum albumin (BSA)) was added and the sample was centrifuged for 5 minutes at 400 x *g*, separating the leucocytes from the microbubbles. The supernatant, containing the microbubbles and captured red blood cells, was subsequently aspirated off. The resultant leucocyte pellet was depleted of RBCs as seen in Figure 2.

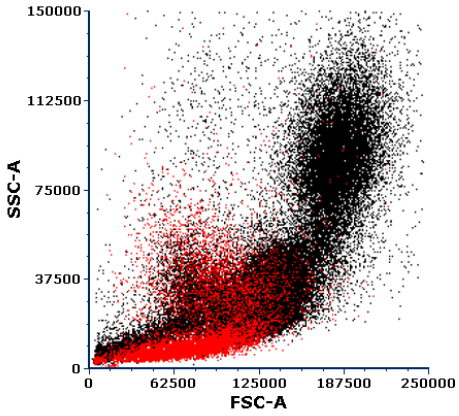
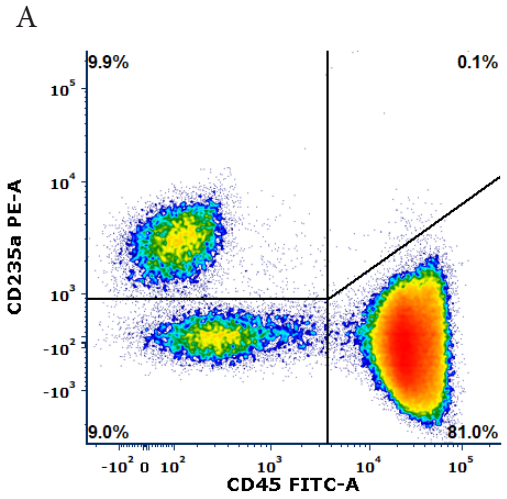


Figure 1. Flow cytometry scatter profile of PMBC sample with a high concentration of contaminating red blood cells (RBCs in red). Red blood cells and their aggregates increase the total cell number and can interfere with gating the lymphocyte population. Flow cytometry was performed on Miltenyi MACSQuant® Analyzer.

Figure 2. Untreated control sample (left) compared to RBC depleted sample (right) post centrifugation and aspiration of buffer.

Cell pellets were resuspended in Separation Buffer and stained with CD45-FITC and CD235a-PE antibodies. Excess stain was removed by washing with 0.5 mL of Separation Buffer. Samples were resuspended in 1 mL of Separation Buffer and RBC depletion efficiency was measured by flow cytometry. As seen in Figure 3, RBC contamination was decreased from 9.9% to 1.3% by treatment with Human Red Blood Cell Microbubbles.



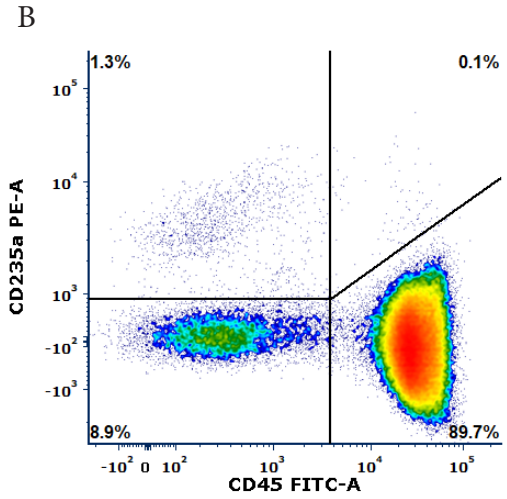


Figure 3. Comparison of red blood cell contamination before (A) and after depletion (B). Flow cytometry performed on Milteny MACSQuant® Analyzer.

In less than 10 min, ~90% of the contaminating red blood cells were removed and the sample was ready for cell sorting or other analysis (Figure 4).

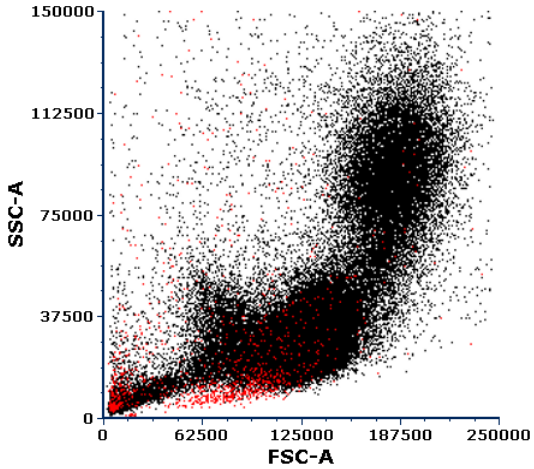


Figure 4. PBMC flow cytometry scatter profile after removal of red blood cell contamination. Residual RBCs in red.

Akadeum Human Red Blood Cell Depletion Microbubbles

To further illustrate the benefit of microbubbles, similar samples of human PBMCs were prepared with a higher level of RBC contamination (~50%) and divided into two equal samples. The first was left untreated and the second was depleted of RBCs with Human Red Blood Cell Depletion Microbubbles. The microbubble fractions showed an average RBC depletion of >95% as compared to the untreated fractions (Table 1). Both samples were then sorted to collect CD19+ B cells.

	% RBC Contamination	% RBC Depletion
Sort 1	47.3%	>99%
Sort 2	44.8%	>99%
Sort 3	49.8%	88%

Table 1. Results of treating three individual samples of RBC contaminated PBMCs with microbubbles prior to sorting.

Figure 5 (below) shows the effect of removal of the RBCs from the samples. Depletion of RBCs resulted in a marked increase in the concentration of leucocytes.

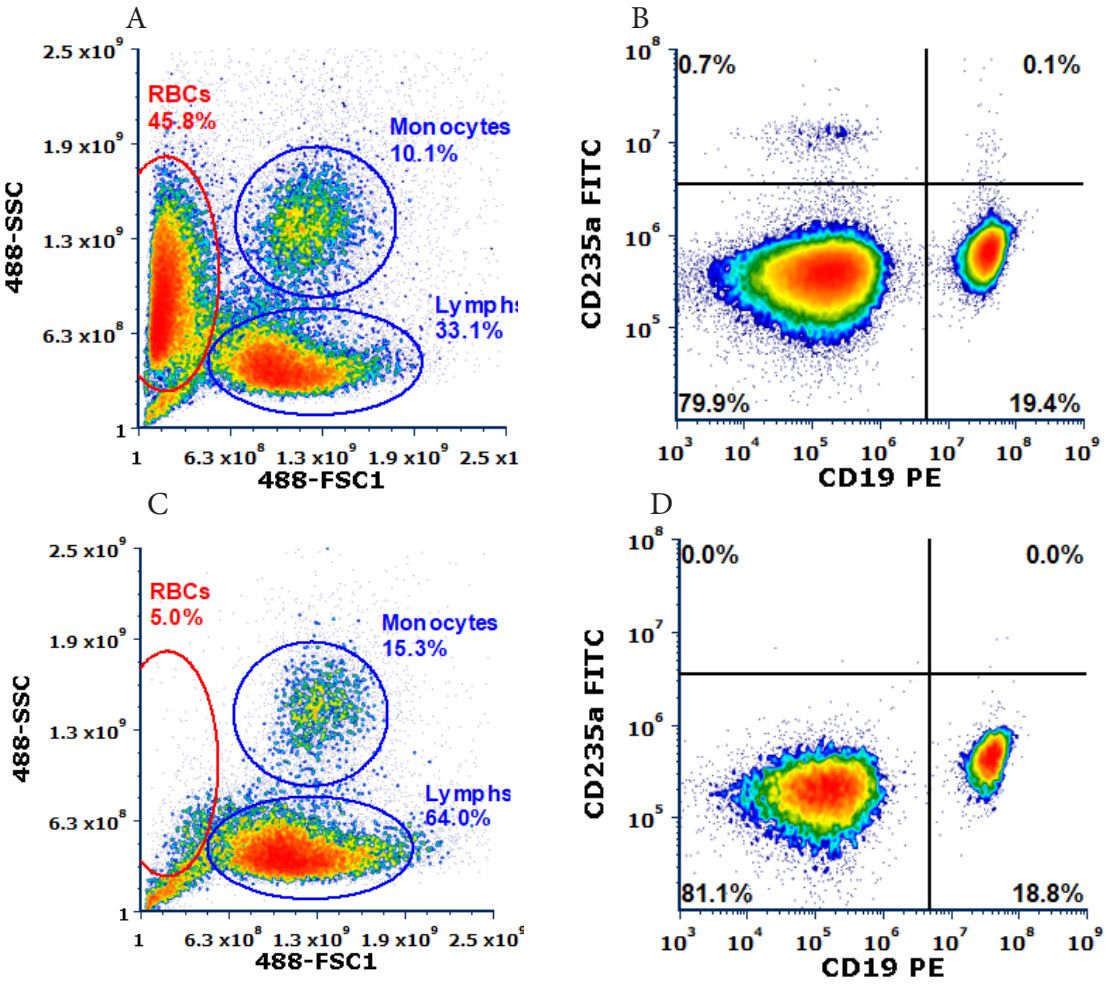


Figure 5. Cell population distributions for the untreated (A&B) and microbubble treated (C&D) PBMC samples. B&D are gated on corresponding Lymphs gate (A &C, respectively).

The sorts were performed at approximately 10,000 events per second and the time to obtain 250,000 CD19+ B cells was recorded. The results are compared between the samples with and without treatment with Human Red Blood Cell Depletion Microbubbles in Table 2.

	WITHOUT MICROBUBBLES		WITH MICROBUBBLES		
	Time w/o RBC clean up	Sort Efficiency	Time w/ RBC clean up	Sort Efficiency	Time Reduction
Sort 1	8.1 min	80%	5.1 min	86%	37%
Sort 2	18.9 min	85%	12.0 min	86-90%	36%
Sort 3	8.0 min	78-80%	5.3 min	81-84%	34%

Table 2. Time to sort 250,000 CD19+ B cells. Samples sorted on Beckman Coulter MoFlo Astrios.

The removal of RBCs from the samples reduced the time of the sort by an average of 36%. Additionally, the microbubble treatment resulted in an increase of sort efficiency of up to 6%. As seen in Figure 5, before sorting, the microbubble treated samples showed a higher percentage of total lymphocytes and more defined populations. This resulted in shorter sort times as there were fewer RBCs contributing to the total event throughput (10,000 events/sec). Furthermore, the reduction in RBCs also improved the sort efficiency as there were fewer events in the stream reducing the occurrence of RBCs in neighboring droplets leading to aborts.

Conclusion

The data demonstrate that Akadeum Human Red Blood Cell Depletion Microbubbles are a simple, effective way to remove unwanted RBCs from samples and can reduce sorting times needed to obtain highly purified cells. Approximately 90% or better removal of RBCs can be achieved in 10 minutes without the need for any additional equipment. This reduced the time necessary to sort human CD19+ B cells from PMBC samples by one-third. During longer sorts, regardless of the cells being collected, the use of Human Red Blood Cell Microbubbles can reduce sort times by hours, saving time and resources, and potentially improving the quality and viability of the sorted cells.

1. Sartor, M., Antonenas, V., Garvin, F., Webb, M., and Bradstock, K.F., Recovery of viable CD34+ cells from cryopreserved hemopoietic progenitor cell products. Bone Marrow Transplantation 2005; 36: 199-204.