Buoyancy Activated Cell Sorting (BACS™) Microbubbles for Lysis and Ficoll Free Processing of Leukopaks

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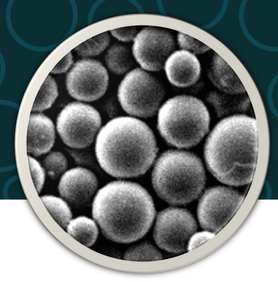


Abstract

Leukopaks are an apheresis product containing large numbers of mononuclear cells. Leukopaks are a vital source material for immunological studies, cell therapy research, and pharmaceutical development. The enrichment of leukocytes from Leukopaks has traditionally required that samples be subjected to either a density gradient separation or red blood cell (RBC) lysis to remove erythrocytes. This pre-processing has made cell isolation leukopak time-consuming, inefficient, and inconsistent. Another challenge of isolating cells from leukopaks is the large volume which can be up to 300 mL or more. To overcome these obstacles, We have developed an approach that is based on our Buoyancy Activated Cell Sorting (BACS™) Microbubbles. Our approach offers density gradient and lysis-free processing of leukopaks. Additionally, our flotation-based cell isolation platform makes it easy to handle large volumes or numerous samples concurrently. Truly untouched cells can be isolated from a leukopak in less than 60 minutes using our quick and easy protocol. Here we report on the isolation of truly untouched T cells and CD4+ T cells directly from leukopak material. T cells were isolated with an average final purity of 93.1% while CD4+ T cells were isolated with an average final purity of 92.2%. In addition to high purity, greater than 90% of T cells and 85% of CD4+ T cells were retained.

Methods

Direct from Leukopak Cell Isolation with BACS™ Microbubbles

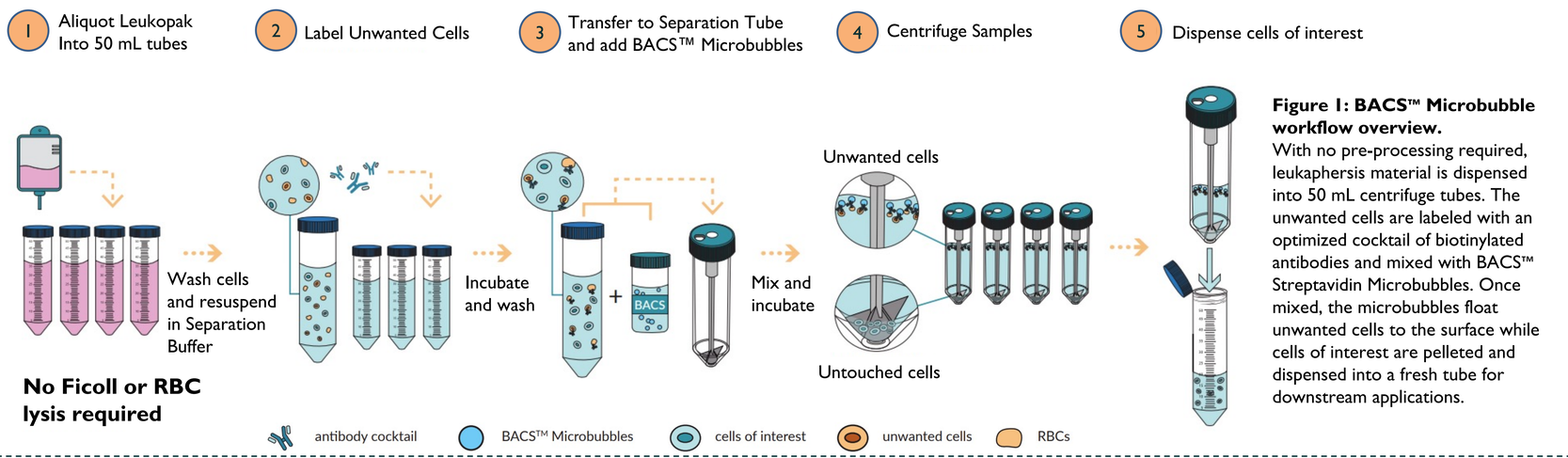


Figure 1: BACS™ Microbubble workflow overview.
With no pre-processing required, leukaphersis material is dispensed into 50 mL centrifuge tubes. The unwanted cells are labeled with an optimized cocktail of biotinylated antibodies and mixed with BACS™ Streptavidin Microbubbles. Once mixed, the microbubbles float unwanted cells to the surface while cells of interest are pelleted and dispensed into a fresh tube for downstream applications.

Results

Flotation-based Cell Isolation For Large Volume Processing

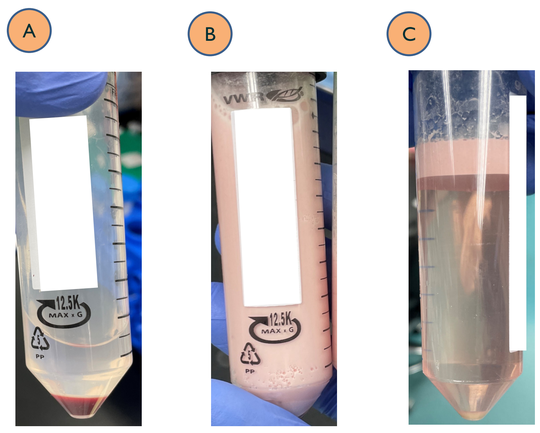


Figure 2: Overview of buoyant cell isolation from leukopak material with BACS™ Microbubbles.
A) Image taken prior to cell isolation depicting 3 billion leukopak cells in a 50-mL conical tube following centrifugation. Cell pellet is colored dark red due to heavy RBC presence. B) Image taken during the cell isolation process depicting 3 billion leukopak cells mixing with streptavidin microbubbles. Streptavidin microbubbles appear white; here the solution is pink in color due to the mixing of white microbubbles and large quantity of RBCs. C) Purified untouched T cells are shown as a white pellet at the bottom of the tube post-isolation. The BACS™ microbubbles are pooled at the top of the tube; note the pink coloration due to effective red blood cell removal.

High Purity, High Yield, High Quality Cell Isolation from Leukaphereis Samples

A

Pan T Cell Isolation Performance Summary

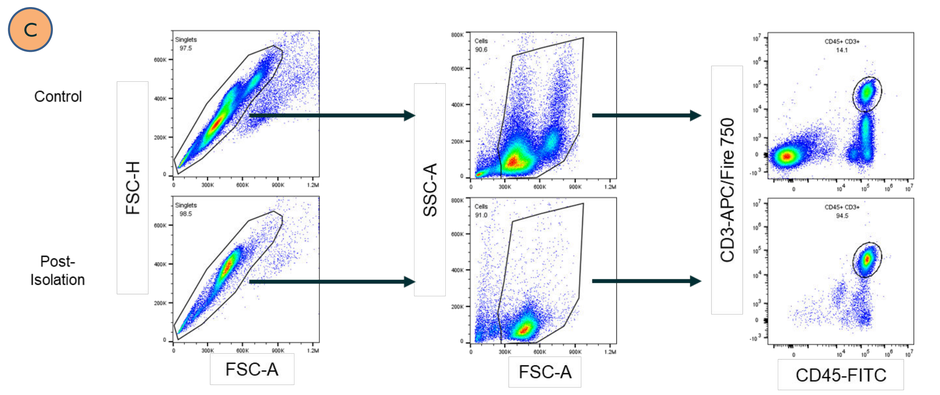
Trial	Initial RBC (% CD235a+)	Initial T Cell (% CD45+ CD3+)	Final T Cell (% CD45+ CD3+)	T Cell Recovery (% of Control)
1	60.2	15.4	93.9	85.5
2	50.1	22.3	94.9	92.6
3	69.4	13.9	93.6	86.8
4	54.3	21.7	90.1	96.9
Average (± SD)	58.5 ± 8.4	18.3 ± 4.3	93.1 ± 2.1	90.5 ± 5.3

B

CD4+ T Cell Isolation Performance Summary

Trial	Initial RBC (% CD235a+)	Initial CD4 T Cells (% CD45+ CD3+ CD4+)	Final CD4 T Cells (% CD45+ CD3+ CD4+)	CD4 T Cell Recovery (% of Control)
1	68.3	11.4	94.0	92.9
2	72.0	8.9	90.8	85.6
3	33.5	19.7	91.2	82.5
4	79.6	4.7	92.7	84.7
Average (± SD)	63.4 ± 20.4	11.2 ± 6.3	92.2 ± 1.5	86.4 ± 4.5

C



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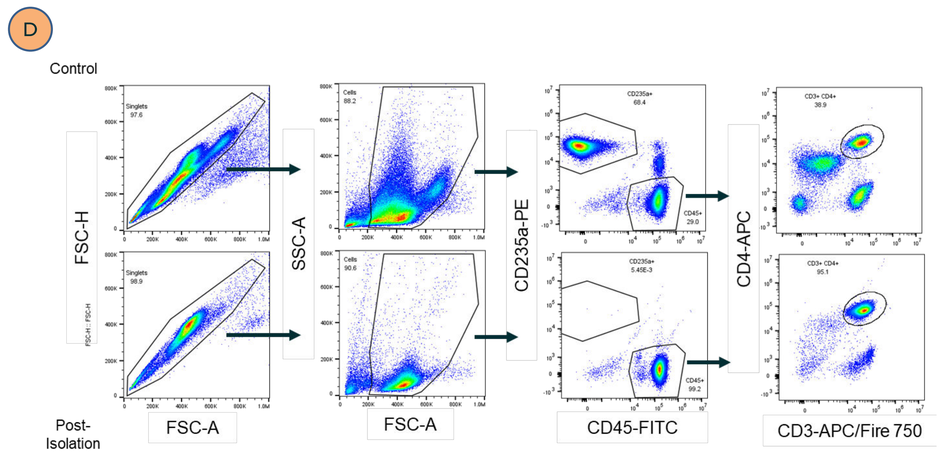

Figure 3: Large volume isolation of immune cells from leukopak using BACS™ Microbubbles.
3B cells from each of four separate donors were processed using the Akadeum Leukopak Pan T Cell Isolation Kit (A,C) or the Leukopak CD4+ T Cell Isolation Kit (B,D). A) Table highlighting purity and yield data associated with pan T cell isolation. B) Table highlighting purity and yield data associated with CD4+ T cell isolation. C) Representative flow cytometry plots depicting pre- (top) and post- (bottom) Pan T cell isolation samples. D) Representative flow cytometry plots depicting pre- (top) and post- (bottom) CD4+ T cell isolation samples.

Particle Analysis Reveals Pristine Purified CD4+ T Cells after BACS™ Microbubble Isolation

A Pre-Isolation

B Post-Isolation

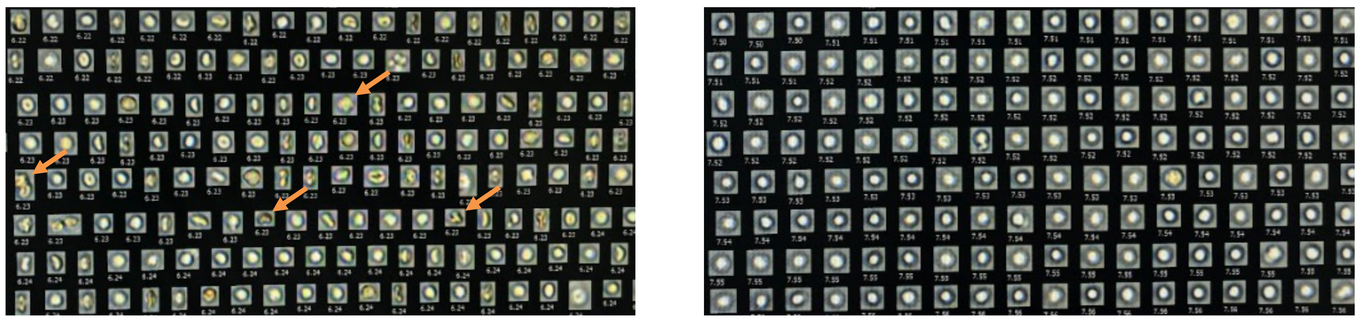

Figure 4: High-Resolution Particle Analysis reveals purified CD4+ T cells.
A FlowCam 8000 Particle Analyzer (Yokogawa Fluid Imaging Technologies) was used to image leukopak cell populations A) before CD4+ T cell isolation and B) after CD4+ T cell isolation using the Akadeum leukopak CD4+ T Cell Isolation Kit. Note the high number of doublets and non-T cells in the control sample (orange arrows).

Conclusions

High Purity, High Yield – Excellent performance with unmatched recovery of T cells

Simple Protocol – No pre-processing required, minimizes sample handling and increases ease-of-use

Gentle – No lysis or gradient required making this the only truly "untouched" product in the market

Easy Handling – New separation tube eliminates aspiration, expands throughput, and enables automation

Robust – Cell isolation that is resistant to donor-to-donor RBC content variability

Scalable – Freed from the restraints of magnets and columns, BACS™ Microbubbles are ideal for large volume cell isolation

Visit our site to learn more

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