Isolation of functional cells straight from buffy coat, LRSC, and Leukopak®

MACS
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Introduction

Enrichment of leukocytes from blood products like buffy coat, leukocyte reduction system chamber (LRSC), or leukapheresis material such as Leukopak® is time consuming and can potentially influence the physiology of the target cells.
Our portfolio of cell separation reagents, the StraightFrom® MicroBeads, were specifically developed to minimize time and handling steps required for cell isolation from these blood products. This is achieved by eliminating the need to perform density gradient centrifugation or erythrocyte lysis prior to isolation, which also preserves cell integrity.
Based on positive selection, CD3⁺ T cells, CD4⁺ T helper cells, CD8⁺ cytotoxic T cells, CD4⁺/CD8⁺ T cells, CD14⁺ monocytes, CD19⁺ B cells, or CD56⁺ NK cells can be isolated from complete buffy coats, LRSCs, or Leukopak® samples within less than 25 minutes with purities above 95%. The positive selection process enables consecutive enrichment of multiple cell populations from a single sample and can be performed in an automated fashion using the MultiMACS™ X.
Using various assays, we show that cells were not activated during separation and functionality was preserved. Ultimately, this optimized magnetic cell separation technology allows the isolation of physiologically relevant cells in a quick and easy manner.

Methods

1 Cell separation based on StraightFrom® MicroBeads – the principle

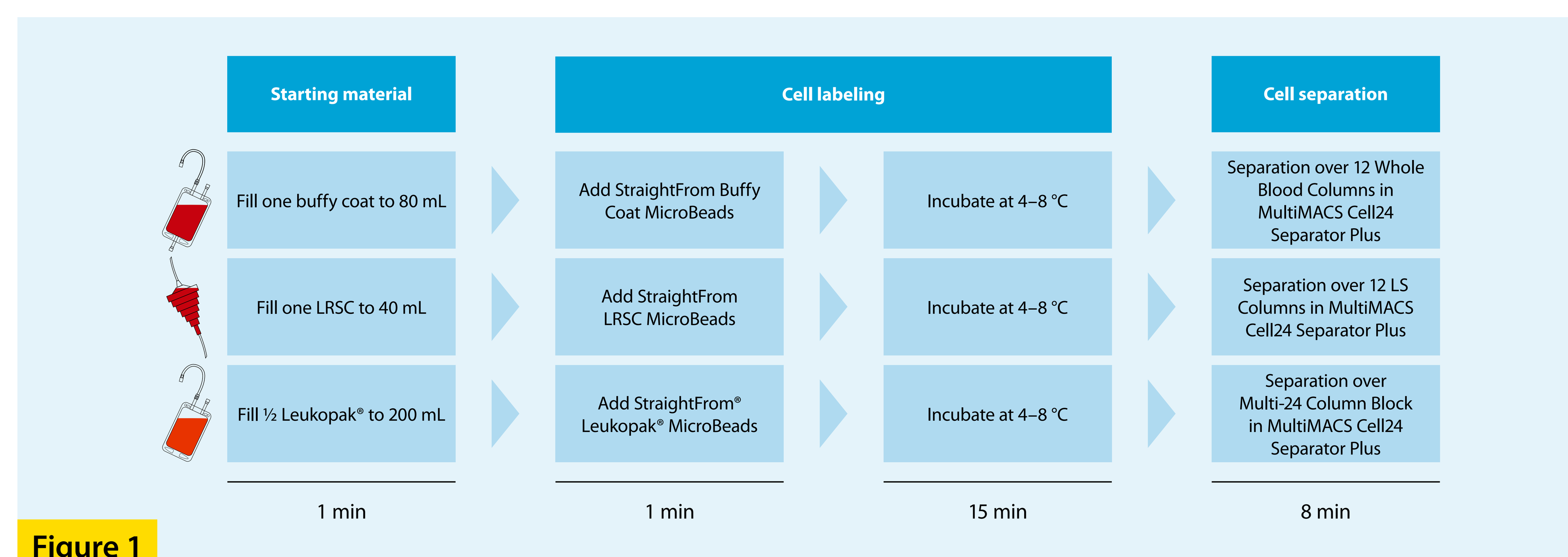

Figure 1

StraightFrom® MicroBeads allow magnetic cell separation directly from buffy coat, leukocyte reduction system chambers (LRSCs), or Leukopaks®. For magnetic labeling, the content of one vial of StraightFrom MicroBeads is added to the cell suspension and incubated for 15 minutes at 2–8 °C. Without any further processing, the cells are then separated directly over 12 Whole Blood or LS Columns or a Multi-24 Column Block placed in the magnetic field of a MultiMACS Cell24 Separator Plus. Processing of a complete buffy coat or LSRC, or 1/2 Leukopak® is done within 25 minutes (fig. 1).

Results

1 Effective enrichment of leukocytes from different blood products with StraightFrom® MicroBeads

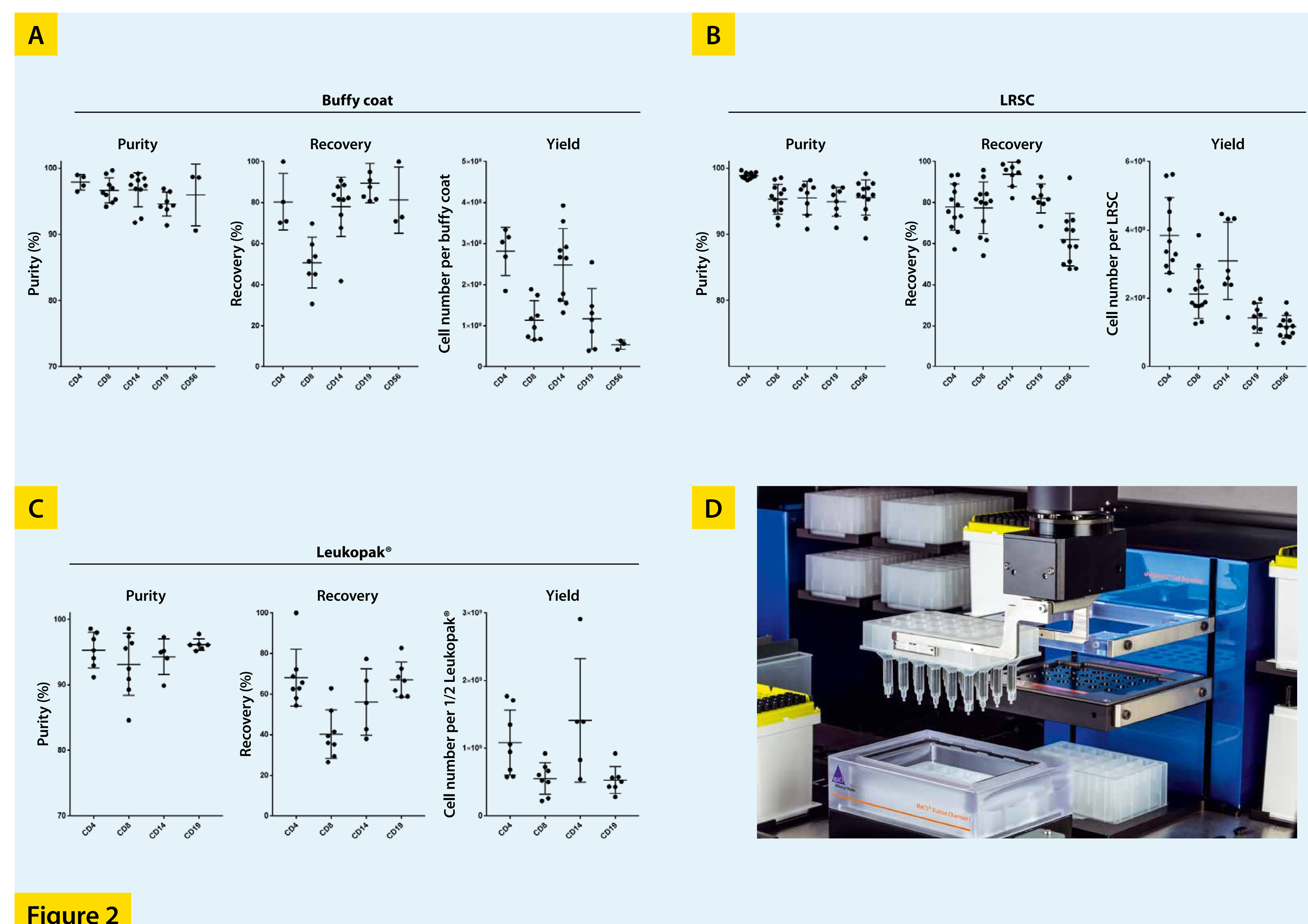

Figure 2

Buffy coat, LRSC samples, or Leukopaks® from healthy donors were used to enrich CD4⁺ T helper cells, CD8⁺ cytotoxic T cells, CD14⁺ monocytes, CD19⁺ B cells, and CD56⁺ NK and NKT cells. Purities and recoveries of enriched cells were assessed by flow cytometry using the MACSQuant® Analyzer 10. Average purities for all cell types was >90%, regardless of the cell source. Recoveries and total cell numbers are shown (fig. 2A–C). Recoveries varied between specificities, donors, and cell sources. These experiments can be fully automated using the MultiMACS X (fig. 2D).

2 Consecutive separation of two leukocyte populations from a single sample

Cell separation step		Purity (%)	Yield per ½ Leukopak® (target cell number)	Percentage of CD4⁺ cells in positive fraction	Percentage of CD8⁺ cells in positive fraction
Enrichment of CD8⁺ cells	Mean	94.0	3.54×10^8	1.80	
	SD	2.8	1.71×10^8	0.83	
Enrichment of CD4⁺ cells	Mean	92.8	1.15×10^9		3.53
	SD	2.1	3.69×10^8		2.46

Table 1

To save precious sample material, it is desirable to have the option to separate multiple cell types from the same sample without splitting it. As a proof of principle, we enriched two cell populations consecutively from commercial Leukopaks® obtained from three different vendors (HemaCare, StemExpress, and AllCells). In particular, 1/2 Leukopaks® were used to enrich CD8⁺ T cells by StraightFrom® Leukopak® CD8 MicroBeads. The negative fraction was then used as starting material for CD4⁺ T cell isolation by StraightFrom® Leukopak® CD4 MicroBeads. Data shown are means obtained with samples from three healthy donors. Cell purities were always >90% after separation, and mean percentages of residual CD8⁺ cells in the enriched CD4⁺ cell fractions amounted to 3.53% (table 1).

3 Functional analysis of CD14⁺ monocytes enriched by StraightFrom® MicroBeads – ROS assay

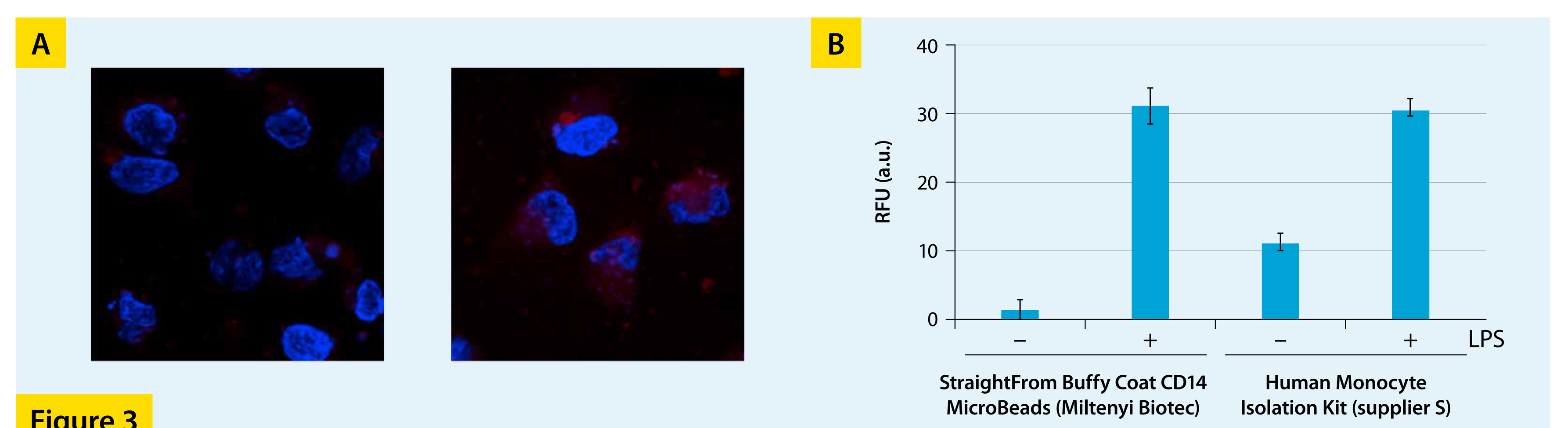

Figure 3

CD14⁺ monocytes were isolated directly from buffy coat by StraightFrom® Buffy Coat CD14 MicroBeads. In a second set of experiments, the Human Monocyte Isolation Kit from another supplier was used to separate monocytes from the same sample. This kit however could not be used directly with buffy coat, but required prior preparation of PBMCs. Unstimulated monocytes enriched by StraightFrom MicroBeads showed no sign of ROS production when analyzed by confocal laser scanning microscopy using the cell-permeable fluorogenic CellROX® Reagent. In contrast, cells enriched from the same donor's PBMCs with reagents from another supplier showed a higher background level of ROS production (fig. 4A). Stimulation of the enriched monocytes with lipopolysaccharide (LPS) induced production of high levels of ROS regardless of the separation method. However, the high background level of ROS production in cells isolated by the Human Monocyte Isolation Kit from the other supplier decreased the sensitivity of the assay by 10-fold (fig. 4B).

4 Functional analysis of CD19⁺ B cells enriched by StraightFrom® MicroBeads – expression of activation markers

Human B cells were enriched from buffy coat using StraightFrom® Buffy Coat CD19 MicroBeads or the Human B Cell Isolation Kit from another supplier. Cells were cultured for 2 days in the absence or presence of CpG ODN 2006 at 5 µg/mL. Expression of activation markers CD69 and CD86 was analyzed by flow cytometry immediately after isolation and after two days of culture. B cells enriched by StraightFrom MicroBeads showed low background expression levels without stimuli also after two days of culture. In contrast, background expression levels for CD69 and CD86 in cells enriched by the reagent from supplier S were significantly higher. Stimulation of B cells with CpG ODN 2006 increased expression levels of activation markers in all samples (fig. 5).

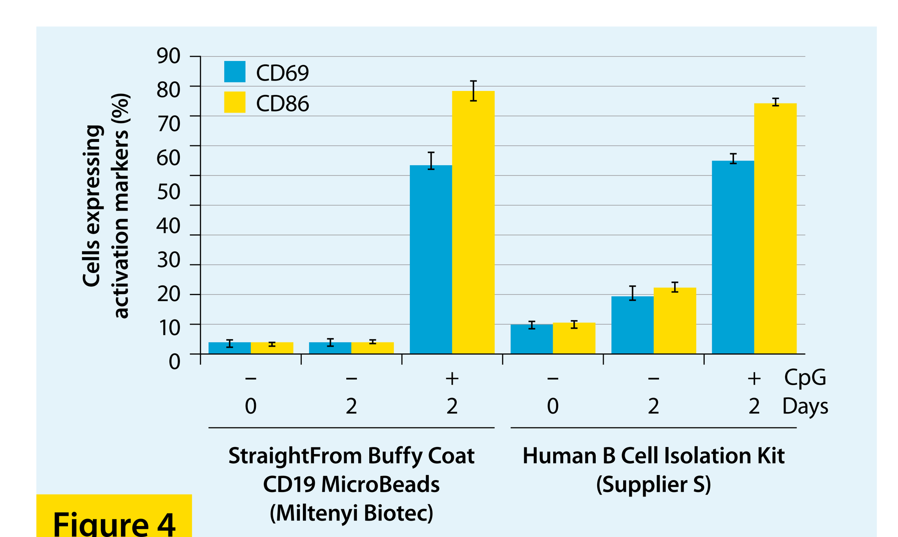

Figure 4

5 Functional analysis of CD19⁺ B cells enriched by StraightFrom® MicroBeads – secretion of Ig

Human B cells were enriched from buffy coat using StraightFrom® Buffy Coat CD19 MicroBeads, human or the Human B Cell Isolation Kit from supplier S. Cells were cultured for 5 days in the absence or presence of CpG ODN 2006, washed in medium, and cultured for another 2 days. Supernatant samples were taken on days 5 and 7. The amount of IgM, IgG, and IgA secreted in the supernatant was evaluated by ELISA. B cells isolated by StraightFrom MicroBeads showed no significant release of Ig without stimulation, while cells isolated with the kit from another supplier secreted considerable amounts of Ig (fig. 6).

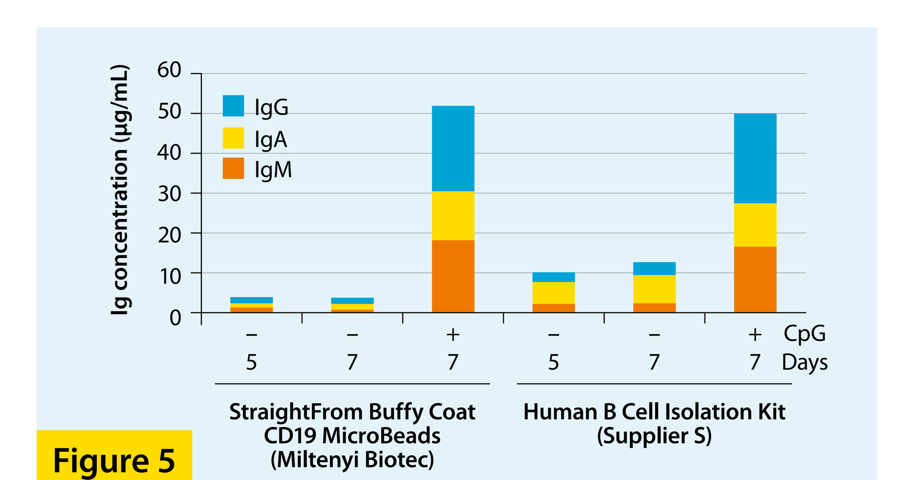

Figure 5

Conclusion

- The StraightFrom MicroBeads for the enrichment of leukocyte subsets from complete buffy coats, LRSCs, or Leukopaks® represent the fastest, simplest, and most convenient method for large-scale cell separation.
- StraightFrom MicroBeads enable rapid cell isolation without the need for density gradients and time-consuming PBMC preparation.
- Consecutive separation of multiple cell types from precious samples is possible.
- The enriched leukocytes are functionally unaffected and can be used directly for downstream applications.

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