A Rapid and Efficient Method for Isolating Plasmacytoid Dendritic Cells from Human Peripheral Blood

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Abstract

Among dendritic cell (DC) subsets, two major populations have been identified, myeloid DC (mDC) and plasmacytoid DC (pDC). Each DC subset comprises only a small fraction of total circulating peripheral blood mononuclear cells (PBMC). Current protocols for their isolation are time consuming, involve multiple steps and often require special equipment. We describe a rapid and simple method for the enrichment by negative selection of pDCs from normal blood that yield excellent cell purities and recoveries. Briefly, PBMCs are isolated by Ficoll-Paque™ PLUS density gradient sedimentation and pDCs are isolated using immuno-magnetic, column-free, negative selection (EasySep®). Our EasySep® technology involves specifically labeling unwanted cells with a cocktail of bispecific tetrameric antibody complexes (TAC) and dextran-coated magnetic particles. Using a magnet, the unwanted cells can then be removed from the unlabeled pDCs. Using our RoboSep® cell separator, the entire separation procedure can be fully automated. Flow cytometric assessment of EasySep® isolated pDCs (Lin^-, $HLA\text{-}DR^+$, $BDCA\text{-}4^+$) demonstrate purities of 93.8% ± 3.8% with cell recoveries of 65.6% ± 16.2% (n=9).

Methods

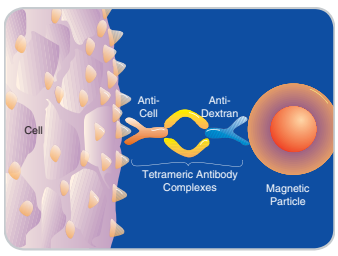


Figure 1: EasySep® labeling of human PBMCs
Unwanted cells are specifically labeled with dextran-coated magnetic particles using bispecific TAC. These TAC recognize both dextran and the cell surface antigen expressed on the unwanted cells. The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep® procedure.

Figure 2: EasySep® procedure for column-free enrichment of plasmacytoid dendritic cells

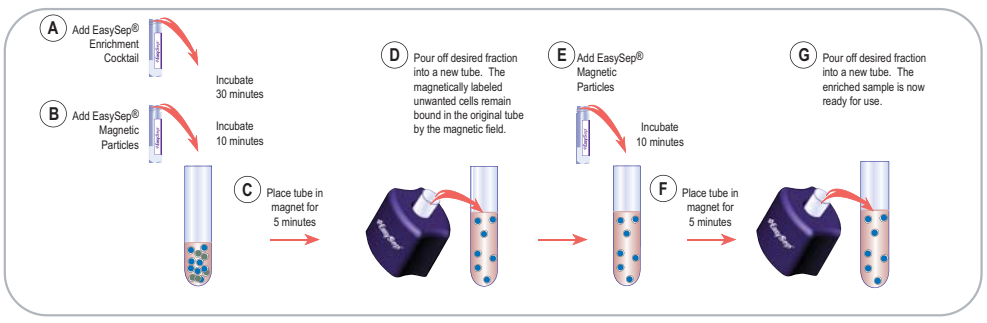
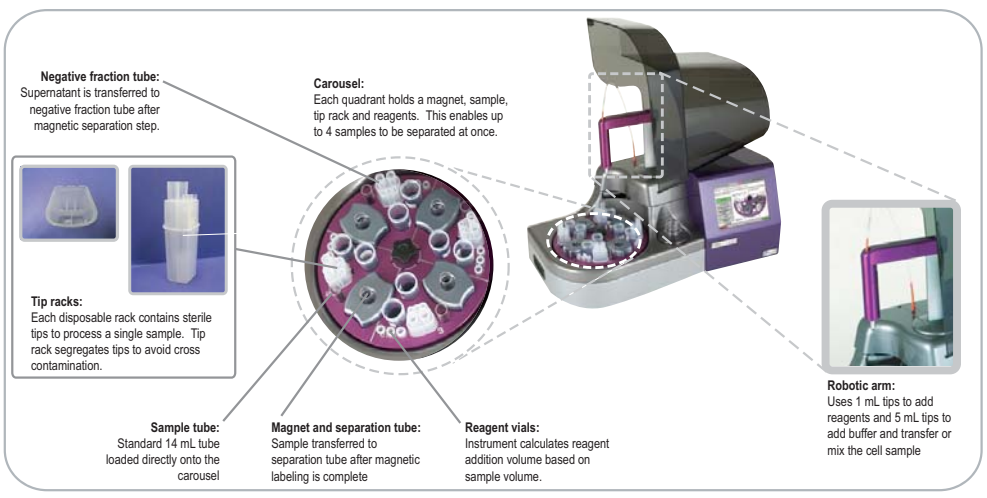


Figure 2: The EasySep® human pDC enrichment kit is designed to isolate untouched pDCs from fresh or previously frozen PBMC samples by depletion of all other non-pDCs (STEMCELL Technologies, Inc. Cat #19062). A) PBMCs are incubated for 30 minutes with the EasySep® pDC enrichment cocktail; B) followed by a 10 minute incubation with EasySep® magnetic particles. C) The tube containing the labeled PBMCs is then placed in an EasySep® magnet for 5 minutes. D) EasySep® enriched pDCs are poured off into a new tube while the magnetically labeled non-pDCs cells are held in the original tube by the EasySep® magnet. E-G) An additional round of magnetic cell separation enables the isolation of highly purified pDCs that are ready to use in functional assays. The entire procedure requires only 60 minutes of incubation time and can be fully automated with the RoboSep® cell separator.

Figure 3: Fully automated enrichment of pDCs using the RoboSep® cell separator



Results

Figure 4: Typical EasySep® and RoboSep® enrichment profiles

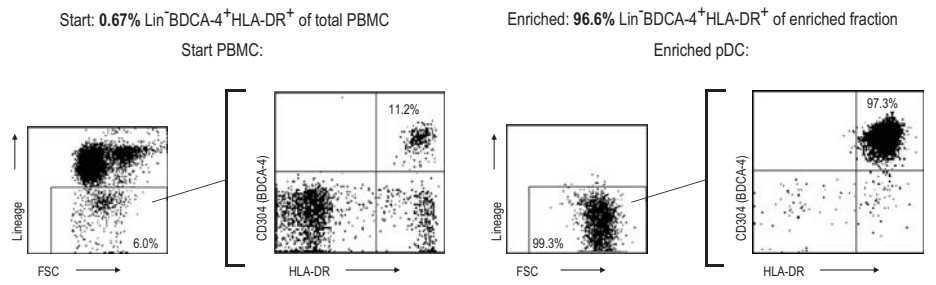


Figure 4: Starting with 0.2 - 0.9% pDC in PBMC, the pDC content of the enriched fraction typically ranges from 87 - 97% purity based on the pDC phenotype of lineage (CD3, CD14, CD16, CD19, CD20, CD34, CD56) negative, HLA-DR positive, and CD304 (BDCA-4) positive.

Table 1: Purity and recovery of $Lin^-HLA\text{-}DR^+CD304^+$ pDCs enriched by negative selection from PBMC using EasySep® or RoboSep®

	n	Purity	Recovery
EasySep®	9	93.8 ± 3.8	65.6 ± 16.2
RoboSep®	5	92.8 ± 2.6	76.8 ± 20.6

Values expressed as means ± SD
Purity and recovery determined by flow cytometry. All samples gated on viable (PI negative) cells.

Figure 5: Virtually all EasySep® enriched pDCs express IFNα following *in vitro* TLR7 stimulation.

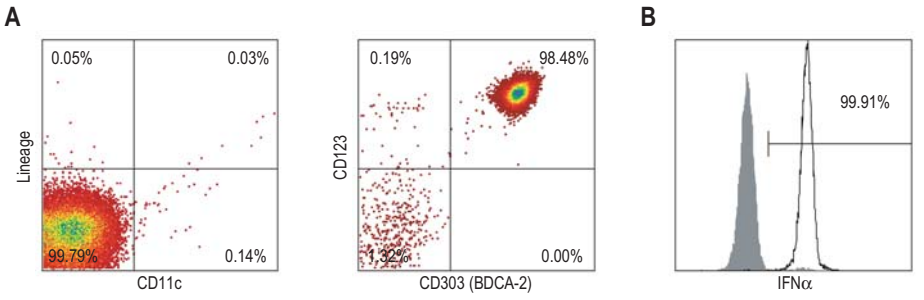


Figure 5: Untouched human pDCs express IFNα following *in vitro* activation. A) EasySep® enriched human pDCs were analyzed for the expression of lineage markers, CD11c, CD123 and CD303 (BDCA-2). B) EasySep® enriched pDCs were cultured for 20 hours in serum-free media supplemented with 5µg/mL Imiquimod-R837, a small synthetic TLR7 ligand. Monensin was added during the final 2 hours of culture followed by flow cytometric assessment of intracellular IFNα. Open and shaded histograms represent staining with anti-human IFNα (clone: MMHA-11) or an isotype control, respectively.

Conclusions

- Untouched human pDCs can be rapidly isolated from PBMC samples using column-free negative selection.
- Excellent purity and recovery can be achieved with the EasySep® and RoboSep® platforms.
- Virtually all EasySep® enriched pDCs express IFNα following *in vitro* TLR7 stimulation with Imiquimod-R837 in serum free media.

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