A RAPID AND SIMPLE METHOD FOR THE ISOLATION OF HIGHLY FUNCTIONAL PLASMACYTOID DENDRITIC CELLS FROM HUMAN PERIPHERAL BLOOD

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ABSTRACT

Plasmacytoid DCs (pDCs) play an important role in antiviral immunity, secreting large amounts of type I and type III interferon (IFN) upon stimulation by live virus or toll-like receptor (TLR) agonists. pDCs comprise only a small fraction of total circulating peripheral blood mononuclear cells (PBMC) and 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 highly functional 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 magnetically-labeled unwanted cells are removed from the unlabeled pDCs. Using our RoboSep® cell separator, the entire 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). Functional assessment of EasySep® and RoboSep®-isolated pDCs demonstrated robust secretion of type I and type III IFN following treatment with TLR agonists and live viruses.

METHODS

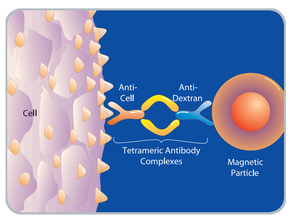
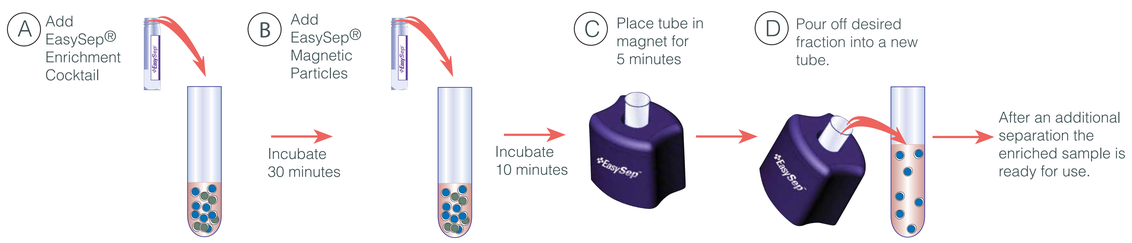


FIGURE 1: EasySep® labeling of human PBMC

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. 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



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. 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 less than 1 hour and can be fully automated with the RoboSep® cell separator.

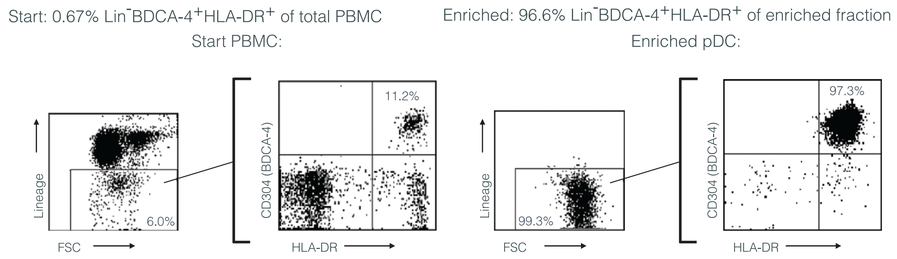
RESULTS

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

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

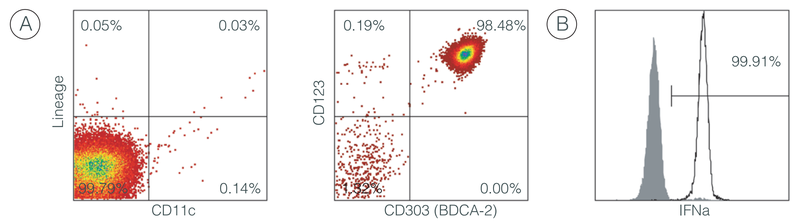
Values expressed as means ± SD
Purity and recovery determined by flow cytometry. All samples gated on viable (PI negative) cells.

FIGURE 3: Typical EasySep® and RoboSep® enrichment profiles



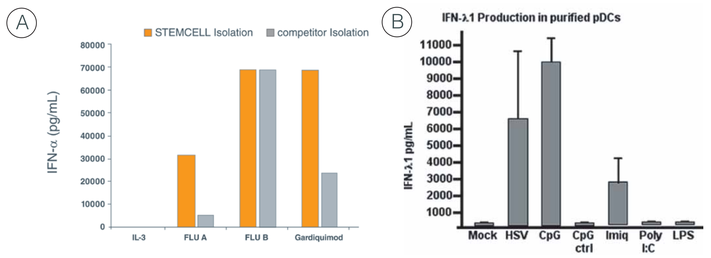
Starting with 0.2 - 0.9% pDCs in PBMC, the pDC content of the enriched fraction typically ranges from 87- 97% purity based on the pDC phenotype of lineage (CD3, CD14, CD16, CD19, CD20, CD34, CD56) negative, HLA-DR positive, and CD304 (BDCA-4) positive.

FIGURE 4: Virtually all EasySep® enriched pDCs express IFNα following *in vitro* TLR7 stimulation



Untouched human pDCs secrete 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.

FIGURE 5: EasySep® or RoboSep®- isolated pDCs secrete Type I andType III IFN following stimulation with various stimuli



A) Compared to competitor isolated pDCs, RoboSep®-isolated pDCs secrete increased levels of IFNα following treatment with type A Influenza or a TLR7 agonist, Gardiquimod™. Data kindly provided by Dr. Michelle Gill from the Department of Pediatrics, University of Texas Southwestern Medical Center. B) EasySep®-isolated pDCs were incubated with various TLR agonists for 24 h and assayed for production of IFNλ1 by ELISA.

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.