A RAPID AND EFFICIENT METHOD FOR THE PRE-ENRICHMENT OF DENDRITIC CELLS FROM HUMAN PERIPHERAL BLOOD SAMPLES

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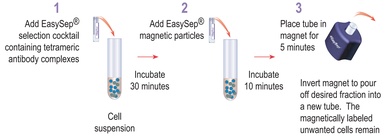
Abstract #219

Summary

Human peripheral blood dendritic cells (DC) are generally defined as either myeloid DC (mDC, phenotypically $Lin^{neg}HLA\text{-}DR^{+}CD11c^{+}CD123^{Lo}$) or plasmacytoid DC (pDC, phenotypically $Lin^{neg}HLA\text{-}DR^{+}CD11c^{neg}CD123^{Hi}$), with each subset having distinct functional attributes and differentiation potential. Their isolation from peripheral blood samples is difficult and time consuming as these cells are extremely rare, comprising around 1.5% of total mononuclear cells. Flow cytometry-based sorting is therefore typically required to obtain highly purified fractions of DC subsets. The objective of this study was to develop a simple pre-enrichment method for peripheral blood dendritic cells that would significantly cut down on subsequent sort time while minimizing cell loss. The resulting single-step method was quick, requiring only 45 minutes to perform from start to finish. Starting with peripheral blood mononuclear cell samples containing an average 1.5 ± 0.6% DC, we found an average 38-fold DC enrichment in the isolated fractions (57 ± 7% $Lin^{neg}HLA\text{-}DR^{+}$, n=15). Average cell recovery values were 90 ± 11% for pDC, and 74 ± 22% for mDC, suggesting minimal cell loss. Minor modifications to the cocktail and protocol also enabled the isolation of highly purified plasmacytoid dendritic cells, with average purities in the enriched samples of 89.4 ± 5.9% (n=11), and cell recoveries of 73.0 ± 11.4%. Both methods were fully automated using a RoboSep® cell separator with true walk-away capability, thereby adding convenience to efficiency.

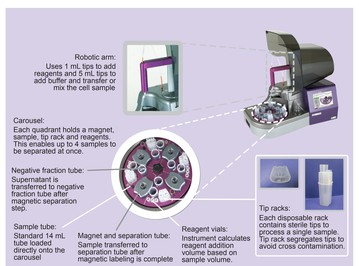
Methods

Figure 1. EasySep® procedure for column-free enrichment of dendritic cells



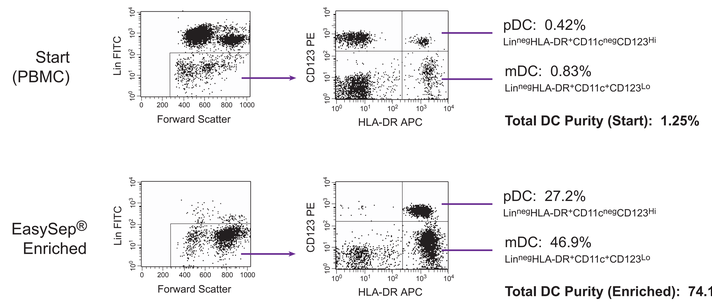
A cocktail of monoclonal antibodies incorporated into tetrameric antibody complexes were used to crosslink unwanted cells in the sample to EasySep® magnetic particles. Antigens targeted were CD3, CD9, CD14, CD16, CD19, CD34, CD56, CD66b, and Glycophorin A. Following incubations times of 30 minutes for the antibody cocktail and 10 minutes for the magnetic particles, the sample was placed in an EasySep® magnet for 5 minutes. Desired cells were recovered by simply pouring off the cell suspension while unwanted cells were held to the walls of the tube where they remained.

Figure 2. Fully automated enrichment of dendritic cells using the RoboSep® automated cell separator.



Results

Figure 3. Pan-DC pre-enrichment using EasySep® and RoboSep®



Dendritic cells were identified using flow cytometry by combining a panel of FITC-conjugated antibodies to lineage markers (Lin FITC: CD3, CD14, CD16, CD19, CD20, CD34, CD56) with APC-conjugated anti-HLA-DR, and PE-conjugated anti-CD123. Dendritic cells were identified as being negative for Lin markers and positive for HLA-DR. Plasmacytoid DC were identified as $CD123^{+}$, and myeloid DC as $CD123^{Lo}$. Myeloid DC were also $CD11c^{+}$, whereas plasmacytoid DC were $CD11c^{neg}$ (data not shown).

Table 1: Results obtained using EasySep® pan-DC pre-enrichment kit starting with PBMC samples.

	n	Total DC Purity (% mDC + % pDC)	% Recovery	
			Myeloid DC	Plasmacytoid DC
EasySep®	15	57.2 ± 7.4	73.7 ± 21.8	93.3 ± 15.4
RoboSep®	12	37.3 ± 14.7	75.7 ± 22.7	82.2 ± 12.9

Figure 4. Single-step plasmacytoid DC isolation by negative selection using EasySep® and RoboSep®

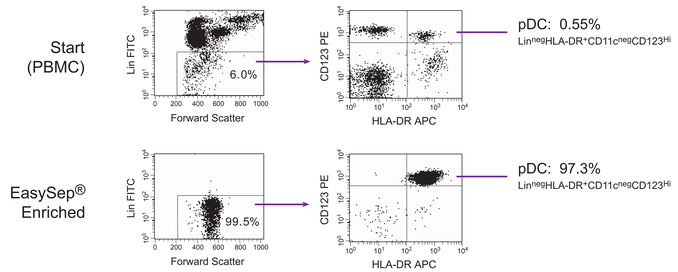


Figure 5. EasySep® enriched pDC are BDCA-4+

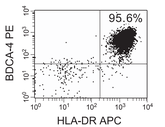


Table 2: Results obtained using EasySep® plasmacytoid DC enrichment kit starting with PBMC samples.

	n	% pDC Purity	% Recovery
EasySep®	11	89.4 ± 5.9	73.0 ± 11.4
RoboSep®	7	89.3 ± 6.0	65.2 ± 19.9

Conclusions

- Human DC can be rapidly isolated from PBMC samples in a single step using column-free negative selection
- Maximum cell recovery can be obtained using either EasySep® or the RoboSep® automated cell separator
- Highly purified BDCA-4+ pDC can easily be isolated by negative selection

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