

A method for rapid isolation of highly purified monocytes using fully automated negative cell selection

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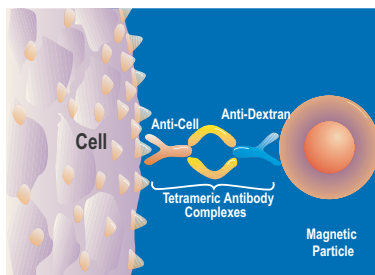
Summary

Monocytes participate in both innate and acquired immune responses. They are responsible for phagocytosis of foreign substance in the body and are capable of killing infected host cells through antibody dependent cytotoxicity. Moreover, monocytes are able to differentiate to macrophages and dendritic cells (DC), serving as scavenger and antigen presenting cells. The preparation of highly purified monocytes for experimentation has traditionally been difficult, requiring multiple steps and many hours of work. In addition, purity and recovery may be low, and purified monocytes are often activated by cell labeling and cross-linking to magnetic particles. We have developed a rapid negative selection method that enables the preparation of highly purified monocytes from human peripheral blood using EasySep[®] column-free immunomagnetic cell separation technology. We also optimized the method for use with the

RoboSep[®] fully automated cell separator. Both the manual and fully automated separation procedures took less than 30 minutes and provided average CD14⁺CD16⁻ monocyte purity above 90% combined with average recoveries above 60%. Culture of purified monocytes in the presence of GM-CSF, IL-4 and LPS led to efficient differentiation into dendritic cells (DC) as identified by the expression of the DC markers CD1a and CD83, and the loss of the monocyte marker CD14. Incubation of the differentiated DC with allogeneic CD4⁺ T cells induced robust CD4⁺ T cell proliferation, which further confirmed that the isolated monocytes were fully competent to differentiate into functional DCs. We report here the successful development of a new one-step method for isolating highly purified and functional monocytes.

Methods

Figure 1. Schematic drawing of EasySep[®] magnetic labeling of human cells



TAC are comprised of two mouse IgG1 monoclonal antibodies held in tetrameric array by two rat anti-mouse IgG1 monoclonal antibody molecules. One mouse antibody recognizes the specific cell surface antigen while the other recognizes dextran on the EasySep[®] magnetic particle.

Figure 2. EasySep[®] procedure for column-free enrichment of human monocytes by negative selection

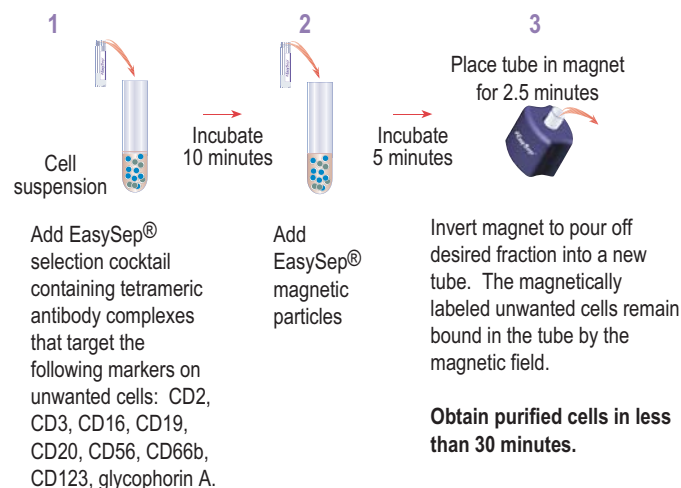
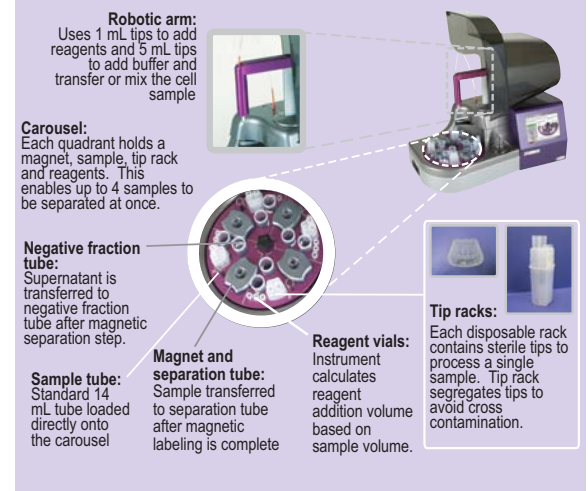


Figure 3. Fully automated enrichment of human monocytes using RoboSep[®]



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Results

Figure 4. EasySep[®] negative selection technology provides highly purified monocyte fractions.

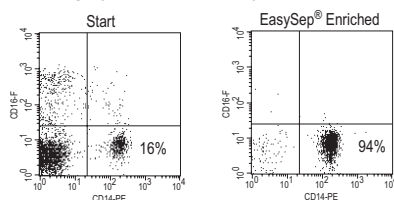
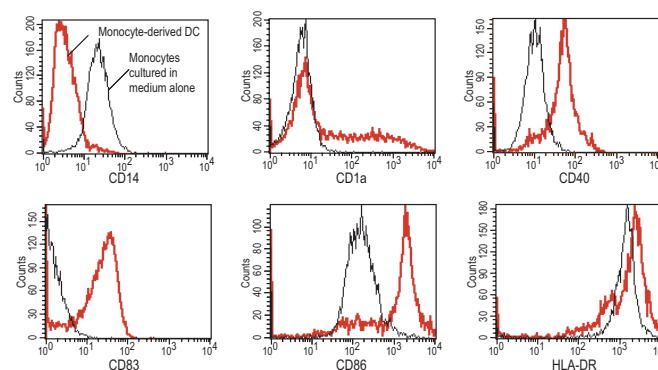


Table 1. Results obtained using EasySep[®] and RoboSep[®] technology for human CD14⁺ CD16⁻ monocyte enrichment from previously frozen mononuclear cells*

	%CD14 ⁺ CD16 ⁻ Cells		Recovery from start		
	Start	EasySep [®]	RoboSep [®]	EasySep [®]	RoboSep [®]
AVERAGE (n=7)	19.8	90.1	90.1	63	71
SD	8.0	3.4	4.6	19	21

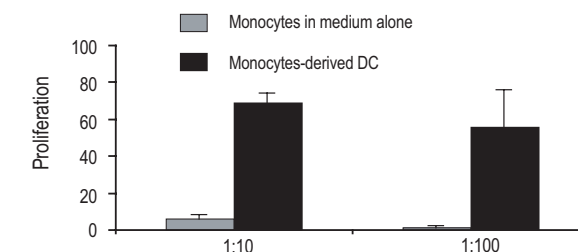
*Mononuclear cells obtained from peripheral blood Leuko Paks collected from normal donors using an apheresis machine were further processed using density centrifugation and cryopreserved in FBS containing 7.5% DMSO prior to use.

Figure 5 Monocytes isolated using EasySep[®] technology can be differentiated to dendritic cells.



Enriched monocytes were differentiated into DC by culturing for 7 days in the presence of GM-CSF, IL-4 and LPS (red line). Enriched monocytes were also cultured in medium alone and served as undifferentiated controls (black line). Cell surface expression of CD14, CD1a, CD40, CD83, CD86 and HLA-DR was measured by flow cytometry.

Figure 6 Dendritic cells derived from monocytes isolated using EasySep[®] technology are capable of inducing T cell proliferation in mixed lymphocyte reactions.



Allogeneic CD4⁺ T cells isolated using EasySep[®] technology were labeled with CFSE and cultured for 7 days in serum-containing medium in the presence of monocytes cultured in medium alone or with cytokines and LPS (monocytes-derived DC) at a cell to CD4⁺ T cell ratio of 1:10 and 1:100. Cell division was analyzed by measuring CFSE in gated viable CD3⁺ cells using flow cytometry. Results are expressed as %cells having undergone ≥ 1 cell division event. Bars are the means of 3 independent experiments, each performed with triplicate culture with Error Bars depicting standard deviations from the mean. No CD4⁺ T cell proliferation was detected when CD4⁺ T cells were cultured in medium alone, whereas stimulation of CD4⁺ T cells with anti-CD3 anti-CD28 coated beads for 7 days resulted in 94% of the cells dividing (data not shown).

Conclusions

- Functional monocytes can be isolated in single step using column-free immunomagnetic EasySep[®] technology.
- Highly purified cells can be obtained in less than 30 minutes.
- The method can be fully automated using the RoboSep[®] cell separator.



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