A simple and rapid method for the isolation of untouched human memory CD8+ T cells

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Abstract

Memory CD8+ T cells are long-lived antigen-specific cells that persist after clearance of infection. Functionally, they are distinguished from naïve CD8+ T cells by their less extensive signal requirement for activation and ability to respond quickly to recall antigens and secrete a broad repertoire of cytokines. In addition, the two populations are phenotypically distinct, with naïve cells expressing CD45RO, and memory cells expressing CD45RA, a marker indicative of previous activation. Current protocols for the isolation of memory CD8+ T cells are time-consuming and require the use of columns. We have developed a new kit for easy and rapid isolation of memory CD8+ T cells from PBMCs by immunomagnetic, column-free, cell separation (EasySep™). Non-CD8+ T cells and CD45RA positive CD8+ T cell subsets are targeted for depletion by bispecific tetrameric antibody complexes crosslinked to dextran-coated magnetic particles. The labeled cells are separated using an EasySep™ magnet and the desired fraction is poured off. The procedure is performed in 30 minutes and can be fully automated using RoboSep™. The mean enrichment purity and recovery of CD45RO−CD45RA−CD8+ T cells is 86% ± 4, and 23% ± 11, respectively. The kit provides a simple and efficient means of isolating untouched human memory CD8+ T cells that are ideal for studies in signal transduction, activation, cytokine expression, and response to infectious disease.

Methods

Preparation of Starting Cell Suspension

A mononuclear cell suspension was prepared from whole blood or buffy coat by Ficoll-Paque PLUS™ density separation. Alternatively, peripheral blood apheresis (Leukopak) cells were used following red blood cell lysis and one or more washes to remove platelets. Start cells were resuspended at 5x10^6 cells/mL in PBS + 2% FBS and 1mM EDTA.

<table>
<thead>
<tr>
<th>Cell population targeted for depletion</th>
<th>Antibodies used in cocktail to target unwanted cells</th>
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<tbody>
<tr>
<td>Non-CD8+ T cells</td>
<td>CD4, CD14, CD16, CD19, CD20, CD34, CD36, CD56, CD61, CD66b, CD123, TCRγδ, GlyA</td>
</tr>
<tr>
<td>Non-memory CD8+ T cells</td>
<td>CD45RA</td>
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Purity Assessment

EasySep™ isolated memory CD8+ T cells were assessed by flow cytometry using a combination of antibodies specific for naïve, effector and memory cells. Memory CD8+ T cells are CD8+CD45RO−CD45RA−.

![FIGURE 1: EasySep™ procedure for column-free enrichment of memory CD8+ T cells from human PBMCs](image)

This procedure can be fully automated using RoboSep™.

![TABLE 1: Antibodies used to deplete non-CD8 T cells and unwanted CD8+ T cell subsets in the EasySep™ Memory CD8+ T cell enrichment kit](table)

![TABLE 2: Purity and recovery of memory CD8+ T cells enriched from PBMC by EasySep™ or RoboSep™](table)

![TABLE 3: Comparison of memory CD8+ T cell isolation protocols using EasySep™/RoboSep™ or the column-based competitor kits](table)

![FIGURE 2: Flow cytometric assessment of memory CD8+ T cells before and after enrichment using EasySep™](image)

Conclusions

- Isolate untouched human memory CD8+ T cells from PBMC in 30 minutes.
- Memory CD8+ T cell isolation can be fully automated with RoboSep™.
- Average purities and recoveries for memory CD8+ enrichments are 85.9% ± 4.4 and 22.7% ± 11.2, respectively.
- EasySep™ isolated memory CD8+ T cells are untouched and are ideal for functional T cell studies.