A fast and simple method for the isolation of untouched human gamma-delta T cells from PBMC

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Summary

T cells expressing the γδT cell receptor (γδTCR) make up a minor subset of human circulating T cells (1-10%). γδT cells are distinct from αβT cells in that they exhibit limited combinatorial diversity of the TCR and recognize non-peptide antigens independent of HLA molecules and antigen-presenting cells. γδT cells share similar characteristics with the cells of innate immune system in that they secrete cytokines and kill target cells without prior antigen exposure or priming. γδT cells display a variety of effector functions. They efficiently kill a range of tumor cells and therefore are being viewed as promising tools in cancer immunotherapy. They are also reactive to microbial agents. γδT cells have been reported to have antigen presenting activity. In addition to innate immune responses, adaptive immune responses such as memory functions and immune regulatory functions have been attributed to γδT cells. Here, we describe a negative selection method to isolate untouched γδT cells from fresh or previously frozen peripheral blood mononuclear cells (PBMC). This method uses immunomagnetic, column-free cell separation technology (EasySep™). Briefly, bispecific antibody complexes are used to cross-link non-γδT cells to dextran-coated magnetic particles. The unwanted cells are then removed using an EasySep™ magnet. Starting with 3±2% γδTCR+ T cells in PBMC, purities of 94±3% (n=23) are achieved. The whole procedure can fully be automated using RoboSep™. The isolated γδT cells are capable of producing interferon-gamma (IFN-γ) when activated with aminobisphosphonates and monocytes. This rapid method for the isolation of γδT cells will assist in the study of γδT cell biology and the development of γδT cell-based immunotherapies.

Methods

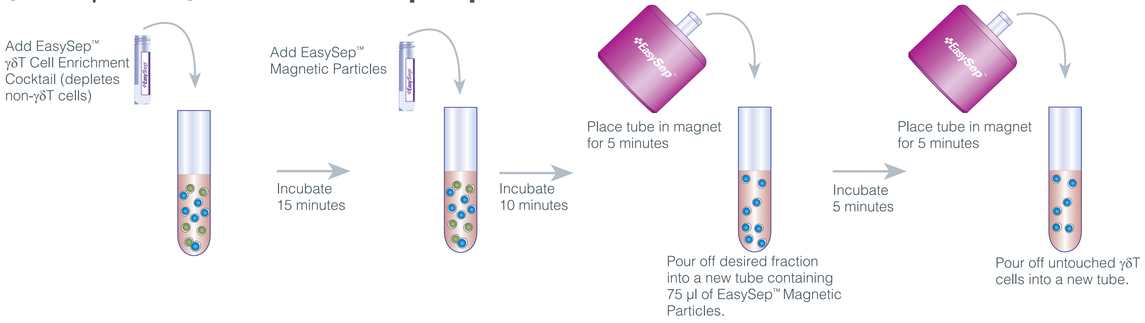
Preparation of Starting Cell Suspension

A single cell suspension of mononuclear cells (PBMC) was prepared from either fresh whole blood or buffy coat suspensions of peripheral blood using Ficoll-paque PLUS™. Alternatively, peripheral blood apheresis (Leukopak PBNC) cells were used following red blood cell lysis and one or more washes to remove platelets. In some experiments, previously frozen mononuclear cells were used. Start cells were resuspended at $5x10^7$ cells/mL in PBS + 2% FBS and 1mM EDTA.

Assessing Enriched Cells

The purity of γδT cells ($CD3^+\gamma\delta TCR^+$) can be measured by flow cytometry after staining with fluorochrome-conjugated anti-CD3, and anti-γδTCR (Clone: IMMU510) antibodies. In addition, intracellular staining of IFN-γ cytokine was assessed after stimulation of cells with either PMA-Ionomycin, or with monocytes in conjunction with the bisphosphonate compound zoledronate.

FIGURE 1: EasySep™ procedure for column-free enrichment of γδT cells ($CD3^+\gamma\delta TCR^+$) from human peripheral blood



Results

FIGURE 2: Phenotypic assessment of human γδT cells ($CD3^+\gamma\delta TCR^+$) enriched using EasySep™

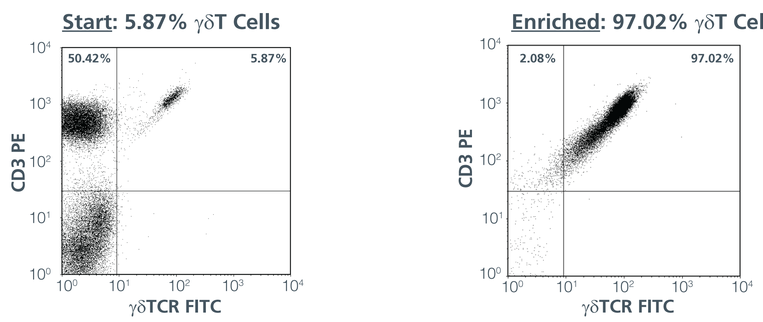
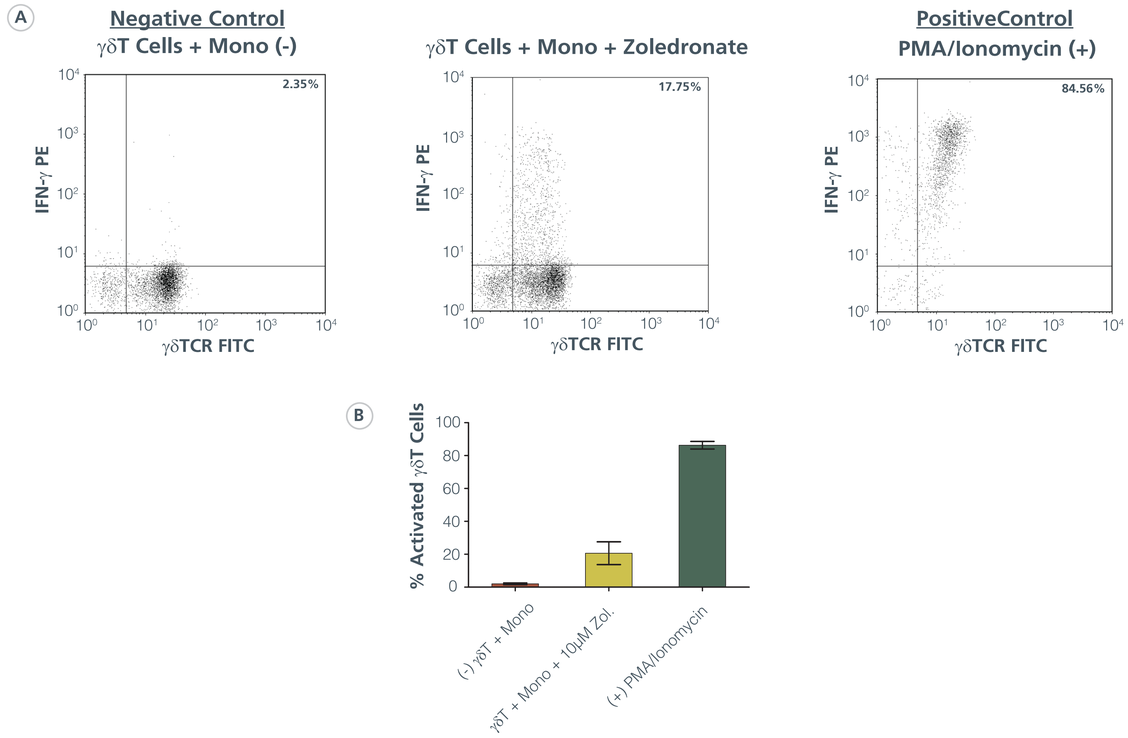


TABLE 1: Purity and recovery of human γδT Cells ($CD3^+\gamma\delta TCR^+$) enriched from peripheral blood by manual Easysep™ or Robosep™

Start	EasySep™			RoboSep™		
% purity	n	% purity	% recovery	n	% purity	% recovery
3 ± 2	23	94 ± 3	29 ± 9	7	92 ± 2	27 ± 6

Purities determined by flow cytometry. Values expressed as mean ± 1 SD.
Viable cells gated using 7AAD staining (7AAD negative gate) and scatter profiles.

FIGURE 3: Enriched human γδT cells express high levels of IFN-γ after stimulation with PMA-Ionomycin or Zoledronate



A representative experiment is shown. A) Intracellular IFN-γ was assessed on human γδT cells ($\gamma\delta TCR^+$) enriched using EasySep™ after overnight stimulation with either PMA-Ionomycin (positive control) or with human $CD14^+$ monocytes (EasySep™ Human CD14 Positive Selection, Catalog #18058) pre-incubated with the bisphosphonate molecule Zoledronate (10 µM). γδT cells co-cultured with monocytes without Zoledronate were used as a negative control. Brefeldin A was added in the last 3 hours of culture. Cells were stained with both anti-γδTCR FITC and anti-IFN-γ PE. Debris and dead cells were gated out using forward vs. side scatter profiles and viability dye eFluor® 660 staining. Activated γδT cells are $\gamma\delta TCR^+IFN\text{-}\gamma^+$. B) Data are the percentage of activated γδT cells (mean ± SD) of triplicate samples for each condition described in A.

Conclusions

- **Untouched human γδT cells ($CD3^+\gamma\delta TCR^+$) can be obtained from PBMC in ~45 minutes.**
- **Enriched human γδT cells have a final purity of 94 ± 3%.**
- **The procedure may be automated with RoboSep™.**
- **EasySep™ human γδT cells are fully functional showing high levels of intracellular IFN-γ after stimulation with PMA-Ionomycin and Zoledronate.**