

# Column-free isolation of untouched human gamma-delta T cells from PBMC in 45 minutes

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## Summary

T cells expressing the  $\gamma\delta$ T cell receptor ( $\gamma\delta$ TCR) make up a minor subset of human circulating T cells (1-10%).  $\gamma\delta$ T cells are distinct from  $\alpha\beta$ T cells in that they exhibit limited combinatorial diversity of the TCR and recognize non-peptide antigens independent of HLA molecules and antigen-presenting cells.  $\gamma\delta$ T cells share similar characteristics with the cells of the innate immune system in that they secrete cytokines and kill target cells without prior antigen exposure or priming.  $\gamma\delta$ T cells display a variety of effector functions. They efficiently kill a range of tumor cells and therefore are viewed as promising tools in cancer immunotherapy. They are also reactive to microbial agents.  $\gamma\delta$ T 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  $\gamma\delta$ T cells.

Here, we describe a negative selection method to isolate untouched  $\gamma\delta$ T 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- $\gamma\delta$ T cells to dextran-coated magnetic particles. The labeled unwanted cells are then removed using an EasySep™ magnet. Starting with  $3 \pm 2\%$   $\gamma\delta$ TCR<sup>+</sup> T cells in fresh PBMC, purities of  $93 \pm 2\%$  (n=17) are achieved. The whole procedure can be fully automated using RoboSep™. The isolated  $\gamma\delta$ T cells are capable of producing interferon-gamma (IFN- $\gamma$ ) when activated with aminobisphosphonates and monocytes. This rapid method for the isolation of  $\gamma\delta$ T cells will assist in the study of  $\gamma\delta$ T cell biology and the development of  $\gamma\delta$ T cell-based immunotherapies.

## Methods

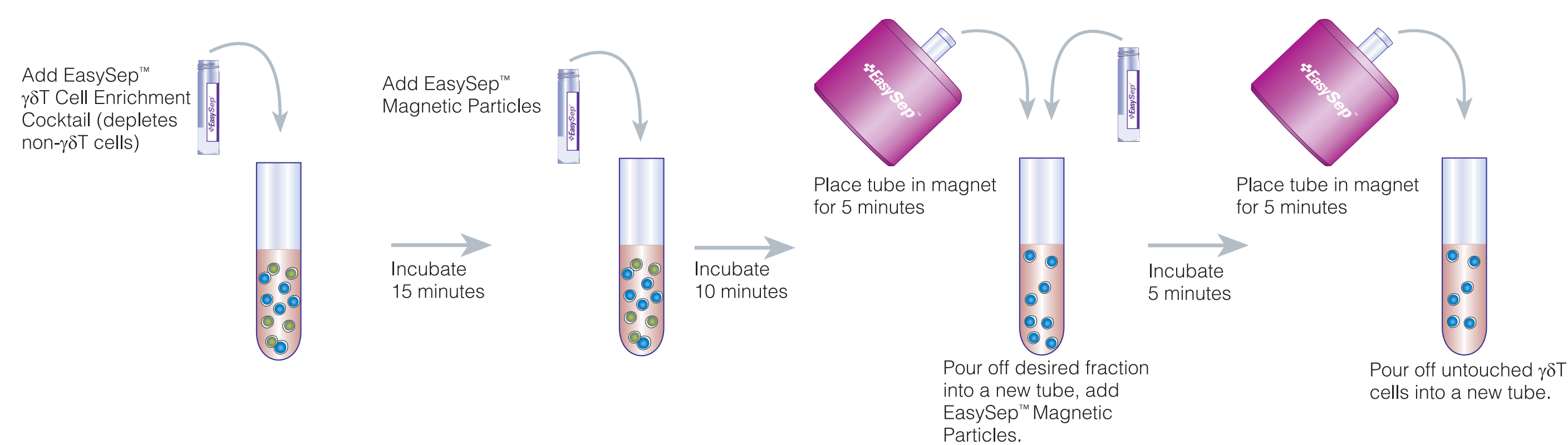
### Preparation of Starting Cell Suspension

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  $5 \times 10^7$  cells/mL in PBS + 2% FBS and 1mM EDTA.

### Assessing Enriched Cells

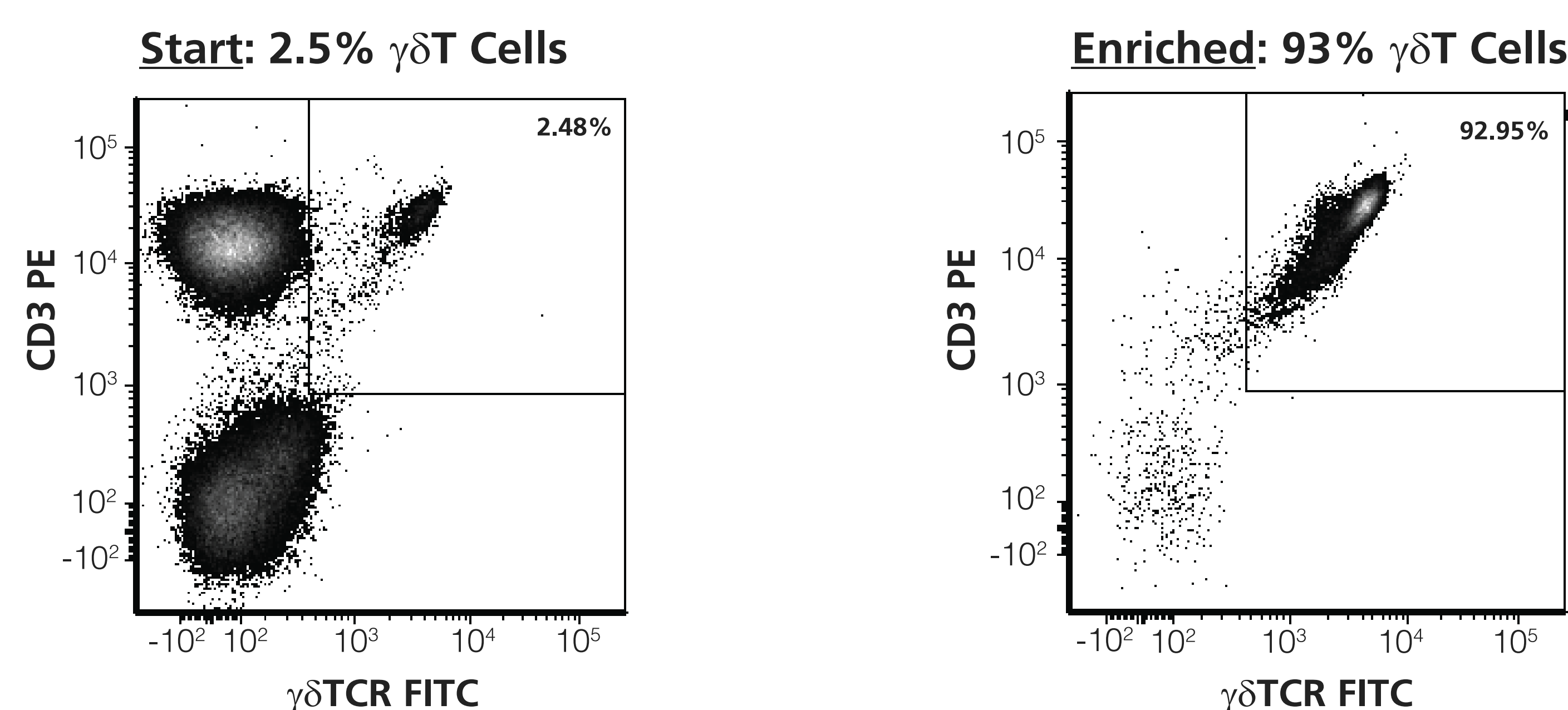
The purity of  $\gamma\delta$ T cells (CD3<sup>+</sup> $\gamma\delta$ TCR<sup>+</sup>) can be measured by flow cytometry after staining with fluorochrome-conjugated anti-CD3, and anti- $\gamma\delta$ TCR (Clones: IMMU510 or 11F2) antibodies. In addition, intracellular staining of IFN- $\gamma$  cytokine was assessed after stimulation of cells with either PMA-Ionomycin, or with monocytes pretreated with the bisphosphonate compound zoledronate.

**FIGURE 1: EasySep™ procedure for column-free enrichment of  $\gamma\delta$ T cells (CD3<sup>+</sup> $\gamma\delta$ TCR<sup>+</sup>) from human peripheral blood**



## Results

**FIGURE 2: Phenotypic assessment of human  $\gamma\delta$ T cells (CD3<sup>+</sup> $\gamma\delta$ TCR<sup>+</sup>) enriched using EasySep™**

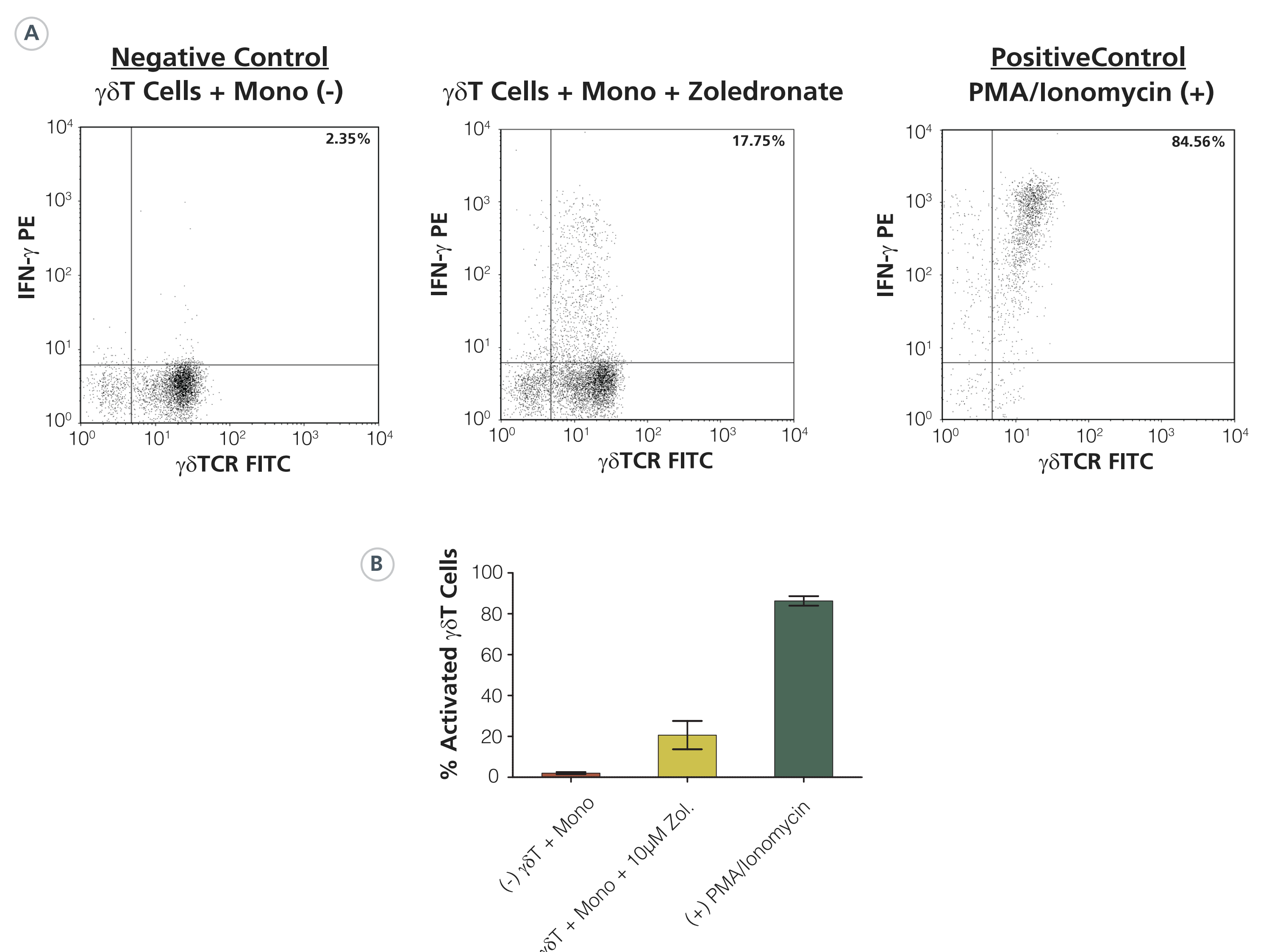


**TABLE 1: Purity and recovery of human  $\gamma\delta$ T Cells (CD3<sup>+</sup> $\gamma\delta$ TCR<sup>+</sup>) enriched from fresh or frozen PBMC by EasySep™**

	START	EasySep™		
	% Purity	n	% Purity	% Recovery
Fresh PBMC	3 ± 2	17	93.5 ± 2.4	53 ± 33
Frozen PBMC		31	81.2 ± 15	28 ± 14

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  $\gamma\delta$ T cells express high levels of IFN- $\gamma$  after stimulation with PMA-Ionomycin or zoledronate**



A representative experiment is shown. A) Intracellular IFN- $\gamma$  was assessed on human  $\gamma\delta$ T cells ( $\gamma\delta$ TCR<sup>+</sup>) enriched using EasySep™ after overnight stimulation with either PMA-Ionomycin (positive control) or with human CD14<sup>+</sup> monocytes (EasySep™ Human CD14 Positive Selection catalog #18058) pre-incubated with the bisphosphonate molecule zoledronate (10 $\mu$ M).  $\gamma\delta$ T 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- $\gamma\delta$ TCR FITC and anti-IFN- $\gamma$  PE. Debris and dead cells were gated out using forward vs. side scatter profiles and viability dye eFluor® 660 staining. Activated  $\gamma\delta$ T cells are  $\gamma\delta$ TCR<sup>+</sup>IFN- $\gamma$ <sup>+</sup>. B) Data are the percentage of activated  $\gamma\delta$ T cells (mean ± SD of triplicate samples) for each condition described in A.

## Conclusions

- **Untouched human  $\gamma\delta$ T cells (CD3<sup>+</sup> $\gamma\delta$ TCR<sup>+</sup>) can be obtained from PBMC in ~45 minutes. (Isolation time for the competitor's negative selection kit is ~90 minutes. Data not shown)**
- **Enriched human  $\gamma\delta$ T cells have a final purity of up to 93 ± 2%.**
- **The procedure may be automated with RoboSep™.**
- **EasySep™ human  $\gamma\delta$ T cells are fully functional showing high levels of intracellular IFN- $\gamma$  after stimulation with PMA-Ionomycin or zoledronate-pretreated monocytes.**