AbsenT™ Negative Selection using the FerroSelect™ Array: Large scale enrichment of untouched T cells or T cell subsets using only one or two monoclonal antibodies

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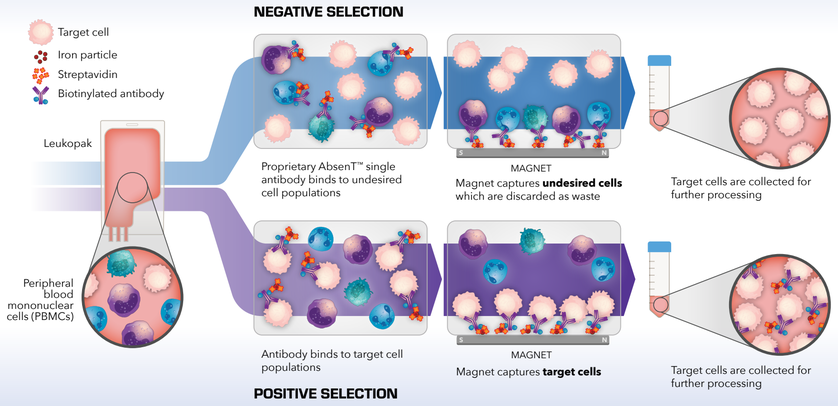
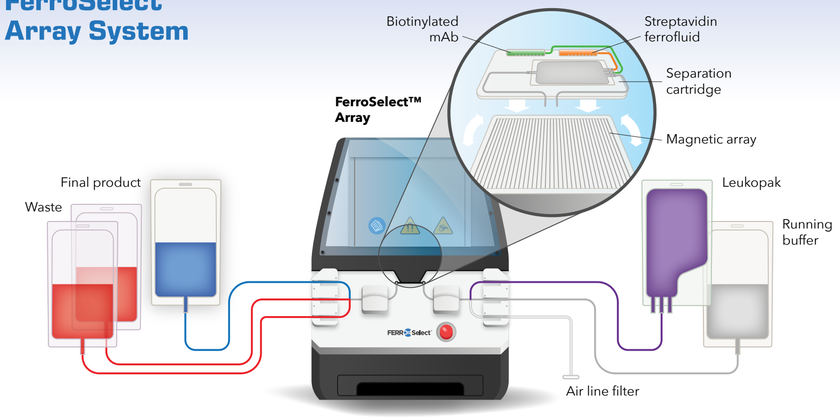
Abstract

Isolation of untouched T cells has long been in demand for clinical immune therapy but has largely been unavailable due to its complexity and the very high cost related to the number of monoclonal antibodies (mAbs) required. Here we present a strategy to enrich untouched T cells by removing unwanted cell types using a single biotinylated monoclonal antibody in combination with highly magnetic nanoparticles (or ferrofluids) conjugated with streptavidin. Using fresh apheresis products from normal donors, an enrichment of anti-CD3+ cells > 84.2 ± 1.6%, was achieved, with viability over 98%. The yield of T cells was in the range of 79.9 ± 10.2%. Moreover, by adding a second antibody to the depletion process, for example either anti-CD4 or anti-CD8 mAb, anti-CD8+ and anti-CD4+ cells, respectively, can be enriched in an untouched state. The selected untouched T cells can be successfully activated and expanded with the FerroSelect™ CD3/CD28 Activation/ Expansion reagents, as demonstrated by measuring cell expansion fold, CD25 expression kinetics and the T cell exhaustion markers PD-1 and Tim-3. In summary, FerroSelect AbsenT™ is a novel system (patent pending) for simple, economical, and rapid cell selection, and provides significant advantages for clinical applications in step with the needs of the cellular immunotherapy community.

Principles of Negative and Positive Selection



FerroSelect™ Array System

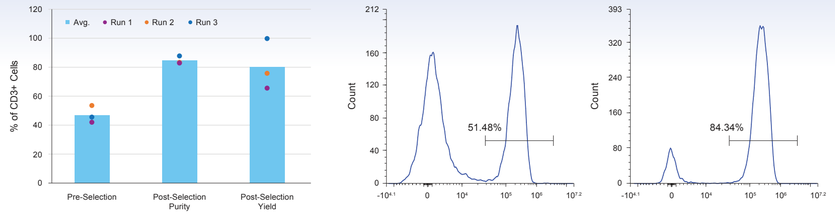


The FerroSelect Array is an automated, functionally closed, cell selection platform for process development and cell therapy manufacturing

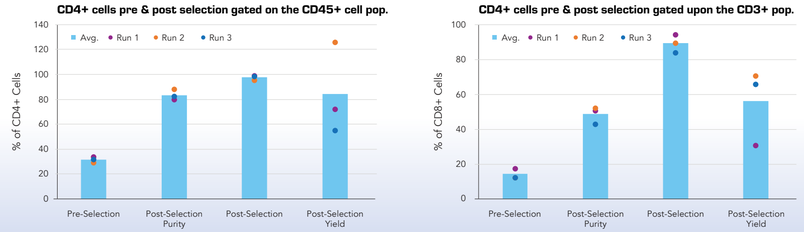
FerroSelect FerroFluid is manufactured with a recombinant human serum albumin coating and labeled with recombinant streptavidin. The shelf life when stored at 2-8 C is in excess of 18 months (with studies continuing). The highly magnetic moment of the particles combined with the planar magnetic array allows for cell isolation without the need for steel-bead columns.

The AbsenT™ kits allow for untouched cells to be collected for further downstream processing

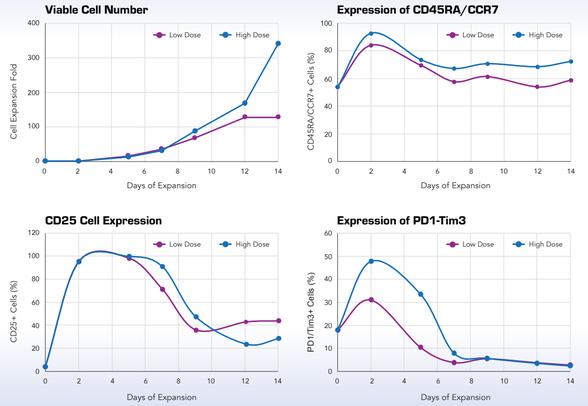
Anti-CD3+ T Cell Enrichment Using AbsenT™ Reagent Kit



Anti-CD4+ & Anti-CD8+ T Cell Enrichment Using the AbsenT™ Reagent Kit Along With Either an Anti-CD4 or Anti-CD8 mAb



T Cell Activation & Expansion After Negative Selection



CD3 Positive cells were selected using the AbsenT™ reagent kit

Cells were activated with mAbs and SA-FF for 30 minutes uisng two different concentrations of Reagents

Low dose: anti-CD3 and CD28 mAbs at 3 ug/mL per mAb with SA-FF at 20 µg/mL

High dose or anti-CD3 and anti-CD28 mAbs at 6 µg/mL per mAb with SA-FF of 40 µg/mL

Cells were subsequently cultured at 5 x 105 cells /ml in PRIME-XV T Cell CDM from Fujifilm containing Interleukin 2 at 10 ng/mL

At each time the cell count and viability, were determined along with the expression of a variety of markers acting as surrogates for the quality of the T cell product obtained

Summary

An innovative cell selection system allowing for the enrichment of T cell and T cell subsets through the depletion of unwanted cells is described. The approach is simple, efficient, and economical, which provides untouched immune cells suitable for downstream applications such as cell expansion and genetic engineering. Using similar approaches, the technology can be applied to the isolations of a variety of other untouched immune cells.

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Cell Selection, Simplified

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