# Workflow Solutions for Human T Cell Isolation and Expansion: EasySep™ T Cell Isolation with Releasable RapidSpheres™ and ImmunoCult™ Human T Cell Activator

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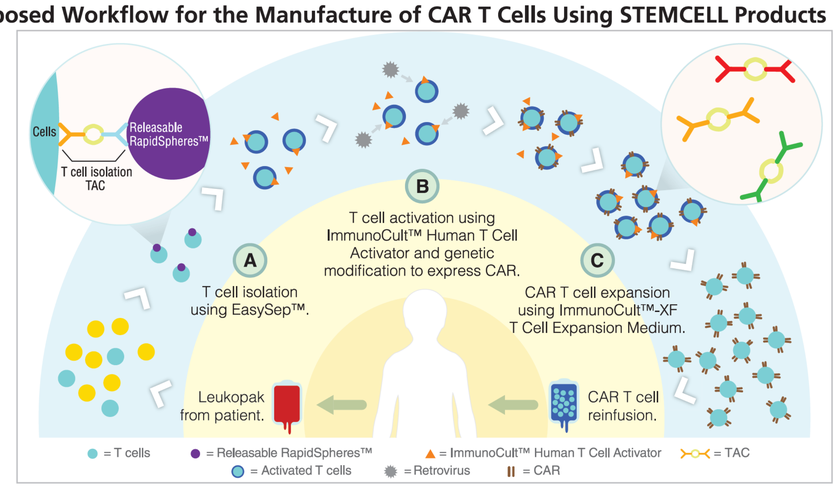
## Abstract

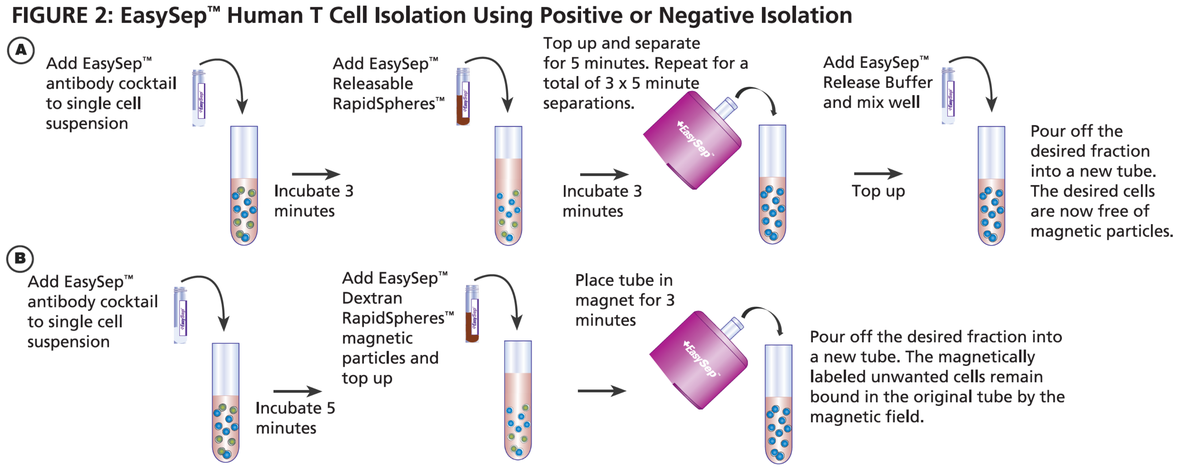
Adoptive cell cancer immunotherapy using patient derived, genetically modified T cells has demonstrated unprecedented success in early clinical trials. Currently, these revolutionary treatment approaches rely on several key steps including the isolation, genetic modification and expansion of patient derived T cells. Specialized reagents are required for each of these critical steps and must be seamlessly integrated into any workflow. We have developed novel reagents for the rapid isolation of highly purified human T cells from leukopheresis samples by a column-free immunomagnetic cell isolation method using EasySep™ Releasable RapidSpheres™, a new type of magnetic particle that can be rapidly released from the isolated cells with a no-incubation, no-wash protocol performed at room temperature. Following their isolation, T cells are genetically modified to express a chimeric antigen receptor and must be expanded to therapeutically relevant numbers. To this end, we have developed an enabling technology using soluble monoclonal antibody complexes for the efficient activation and expansion of human T cells that can be incorporated into any T cell manufacturing workflow. Benefits of our novel ImmunoCult™ supplement include robust T cell expansion and the supplement is stable for at least two years at 2 - 8° C. Furthermore, the ImmunoCult™ supplement provides a gentle activation stimulus resulting in high viability of expanded T cells without skewing the ratio of $CD4^+$ and $CD8^+$ T cells and has been optimized for use with our new serum- and xeno-free ImmunoCult™-XF T Cell Expansion Medium. Taken together, we have developed a streamlined workflow solution for the isolation, activation and expansion of human T cells in serum-free culture conditions to enable this exciting new field. STEMCELL Technologies manufactures products under an ISO 13485 medical device quality management system and is actively pursuing higher compliant manufacturing of key reagents for cellular therapy applications.

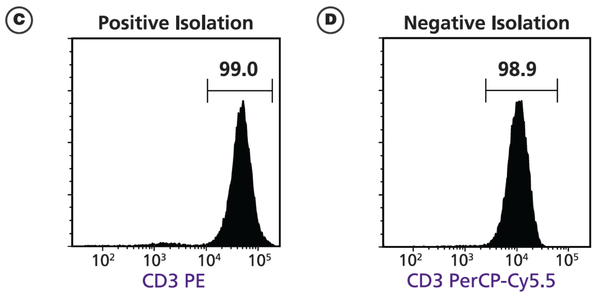
**FIGURE 1: Proposed Workflow for the Manufacture of CAR T Cells Using STEMCELL Products**




**A)** EasySep™ human T cell isolation using EasySep™ Releasable RapidSpheres™; **B)** T cell activation using soluble ImmunoCult™ Human T Cell Activator and gene modification to express CAR construct; and **C)** expansion of CAR T cells in xeno-free and serum-free ImmunoCult™ T Cell Expansion Medium.

**FIGURE 2: EasySep™ Human T Cell Isolation Using Positive or Negative Isolation**




**A)** EasySep™ human T cell positive isolation protocol using EasySep™ Releasable RapidSpheres™. **B)** EasySep™ human T cell negative isolation protocol using EasySep™ Dextran RapidSpheres™. **C)** Following EasySep™ human T cell positive isolation from PBMCs, the $CD3^+$ cell content is typically 99.2 ± 0.2% (mean ± SD using the EasySep™ magnet). **D)** Following EasySep™ human T cell negative isolation from PBMCs, the $CD3^+$ cell content is typically 96.7% ± 1.5% (mean ± SD using the EasySep™ magnet).

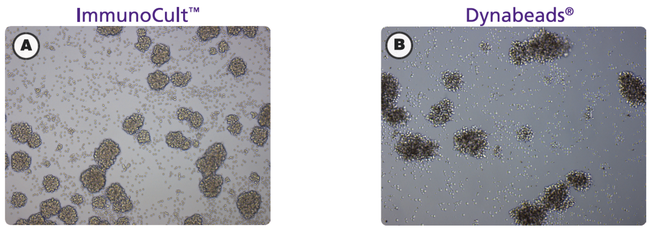
## Summary

- EasySep™ Isolated T cells can be activated and expanded with ImmunoCult™ Human T Cell Activator in serum-free and xeno-free ImmunoCult™ Human T Cell Expansion Medium
- ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator and ImmunoCult™ Human CD3/CD28 T Cell Activator provides a gentle activation stimulus and robust expansion of viable human $CD4^+$ and $CD8^+$ T cells
- EasySep™ and ImmunoCult™ reagents can be easily incorporated into any workflow for the isolation, activation and expansion of human T cells

**TABLE 1: Human T Cell Activation Reagent Comparisons**

| Activation Reagent | Composition | Mechanism of Action | Stability | Method of Use |
|---|---|---|---|---|
| ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator | Soluble monospecific tetrameric antibody complexes against human CD3, CD28 and CD2 | Two monoclonal antibodies in a tetrameric antibody complex are specific for the same cell surface antigen (eg. CD3, CD28 or CD2). The two monoclonal antibodies in the antibody complex cross link cell surface antigens, resulting in T cell activation. Unbound antibody complexes can be washed away or diluted during the course of T cell culture and expansion. | 2 years at 2 - 8°C | 25µL/mL of reagent to 1 x $10^6$ cells/mL |
| ImmunoCult™ Human CD3/CD28 T Cell Activator | Soluble monospecific tetrameric antibody complexes against human CD3 and CD28 | | | |
| Dynabeads® Human T-Activator CD3/CD28 | 4.5µm magnetic beads with immobilized CD3 and CD28 antibodies | Covalently immobilized antibodies against CD3 and CD28 on a cell sized magnetic bead crosslink CD3 and CD28 upon binding to a T cell, resulting in cell activation. Magnetic particles must be removed at the end of culture from the expanded T cells. | 2 years at 2 - 8°C | 3:1 beads to cell ratio at 1 x $10^6$ cells/mL |

**FIGURE 3: Activated Morphology of Stimulated Human T Cells**




EasySep™ isolated human T cells were cultured for 3 days with **A)** ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator or **B)** Dynabeads® Human T-Activator CD3/CD28 (200X magnification).

**FIGURE 4: ImmunoCult™ Human T Cell Activation and Expansion Experimental Workflow**

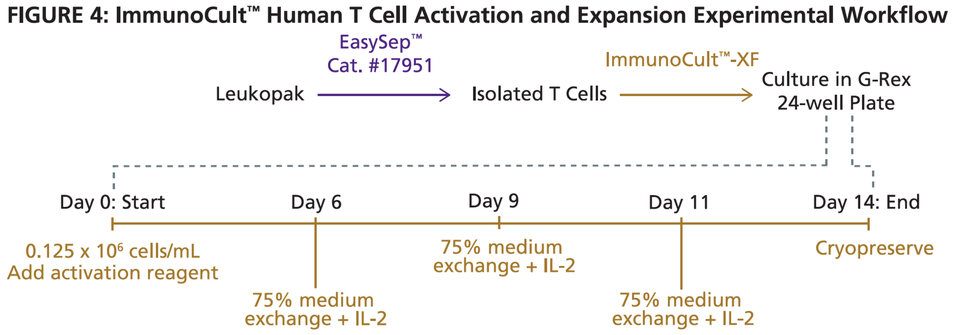
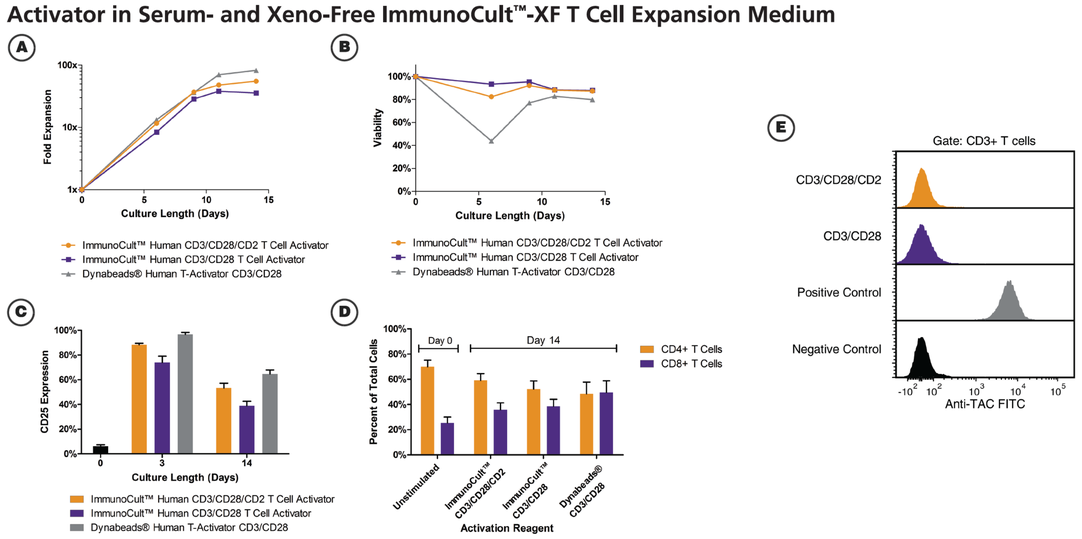



**FIGURE 5: Activation and Expansion of EasySep™ Isolated Human T Cells with ImmunoCult™ Human T Cell Activator in Serum- and Xeno-Free ImmunoCult™-XF T Cell Expansion Medium**




**A)** Fold expansion of EasySep™ isolated human T cells stimulated with activation reagents and cultured in ImmunoCult™-XF T cell expansion medium. **B)** ImmunoCult™ Human T Cell Activator stimulated T cells maintained high viability. **C)** ImmunoCult™ Human T Cell Activator stimulated T cells provide a gentle activation stimulus. **D)** ImmunoCult™ Human T Cell Activators provide robust expansion of $CD4^+$ and $CD8^+$ T cells. **E)** Flow cytometric assessment of residual bound ImmunoCult™ Human T Cell Activator following T cell expansion.