T CELL THERAPY RESEARCH

Isolate, Activate, and Expand Human T Cells

Production of human T cells for cell therapy is a complex, multi-step process. There are many opportunities for optimization to obtain maximum yield while retaining desired end phenotype and function. Explore reagents for optimized human T cell therapy research.

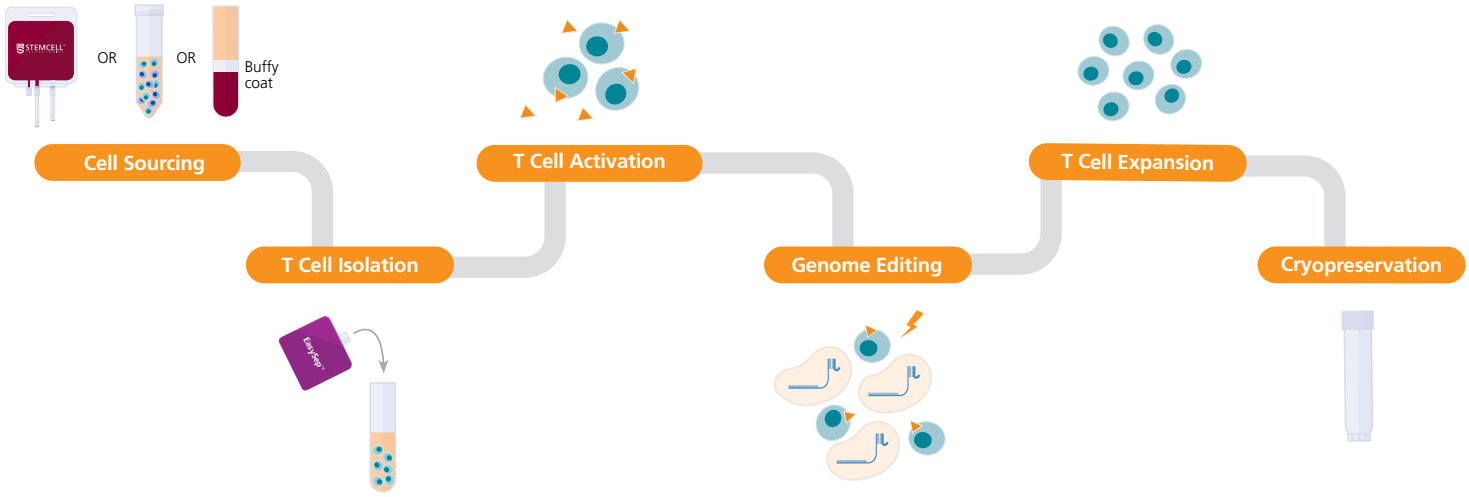


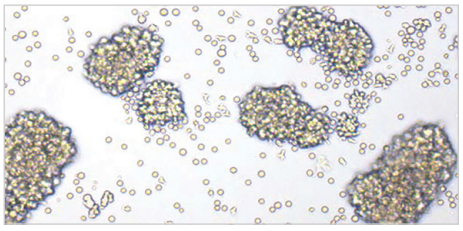
Figure 1. Human T Cell Therapy Research Workflow

Key Technologies for T Cell Therapy Research

EasySep™ Immunomagnetic Cell Separation

Isolate highly purified human T cells in as little as 8 minutes.

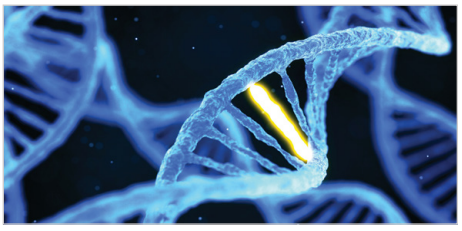
www.EasySep.com



ImmunoCult™ Cell Activation and Expansion

Activate and expand T cells without the use of serum, beads, or plate-bound antibodies.

www.ImmunoCult.com



ArciTect™ CRISPR-Cas9 Genome Editing

Perform high-efficiency editing of T cells using CRISPR-Cas9 ribonucleoprotein (RNP) complexes.

www.stemcell.com/ArciTect

Isolate highly purified T cells from Leukopaks or PBMCs using EasySep™

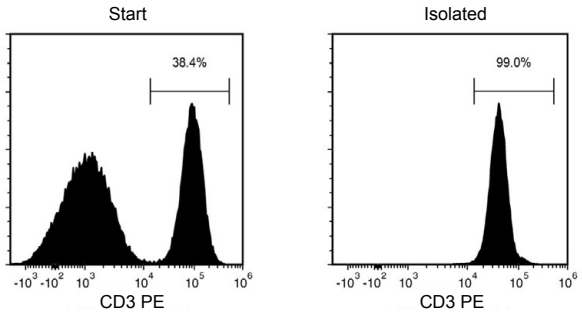


Figure 2. T Cells are Highly Purified When Isolated with EasySep™ Release Human CD3 Positive Selection Kit

Starting with human PBMCs, the CD3+ cell content of the fraction isolated using the EasySep™ Release CD3 Positive Selection Kit (Catalog #17751) is typically 98.7 ± 0.9% (mean ± SD using the purple EasySep™ Magnet).

Activate T cells with ImmunoCult™ Human T Cell Activators

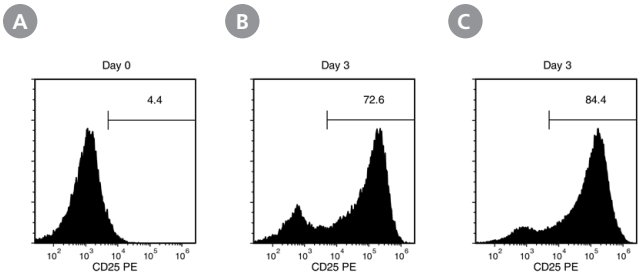


Figure 3. T Cells are Activated When Stimulated with ImmunoCult™ Human CD3/CD28 or CD3/CD28/CD2 T Cell Activator

EasySep™ isolated T cells were cultured on day 0 with either ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) or ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #10970) in ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981). Cells were gated on CD4+ T cells and CD8+ T cells and T cell activation was assessed by CD25+ expression on day 0 and day 3. At the start of culture, the CD25+ cell population was (A) 5.63 ± 2.4% (mean ± SD). After three days of activation, the CD25+ cell population was (B) 75.4 ± 13.8% (mean ± SD) when activated with ImmunoCult™ Human CD3/CD28 T Cell Activator and (C) 88.8 ± 3.2% (mean ± SD) when activated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator.

Product	Catalog #
Human Peripheral Blood Leukopak, Fresh*	70500
Human Peripheral Blood Mononuclear Cells, Frozen	70025
EasySep™ Human T Cell Isolation Kit	17951
EasySep™ Human CD4+ T Cell Isolation Kit	17952
EasySep™ Human CD8+ T Cell Isolation Kit	17953
EasySep™ Release Human CD3 Positive Selection Kit	17751
EasySep™ Release Human CD4 Positive Selection Kit	17752
cGMP, ImmunoCult™ Human CD3/CD28 T Cell Activator	100-0784
cGMP, ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator	100-0785
ArciTect™ Cas9 Nuclease	76002
ArciTect™ crRNA	76010 / 76011 / 76012
ArciTect™ tracrRNA kit	76016 / 76017 / 76018
ArciTect™ Human HPRT Positive Control Kit	76013
ImmunoCult™-XF T Cell Expansion Medium	10981
cGMP, ImmunoCult™-XF	100-0956
StemSpan™ T Cell Generation Kit	09940
CryoStor® CS10, CS5 and CS2	07930 / 07933 / 07932
Hypothermosol® FRS	07935
Cytokines: IL-2 ACF, IL-7 ACF and IL-15 ACF	78193 / 78196 / 78218
Anti-CD25 Antibodies	60153 / 60158
Anti-CD3 Antibodies	100-0285 / 60127 / 60011

*Only available in select territories

Genetically modify human T cells using the ArciTect™ CRISPR-Cas9 system

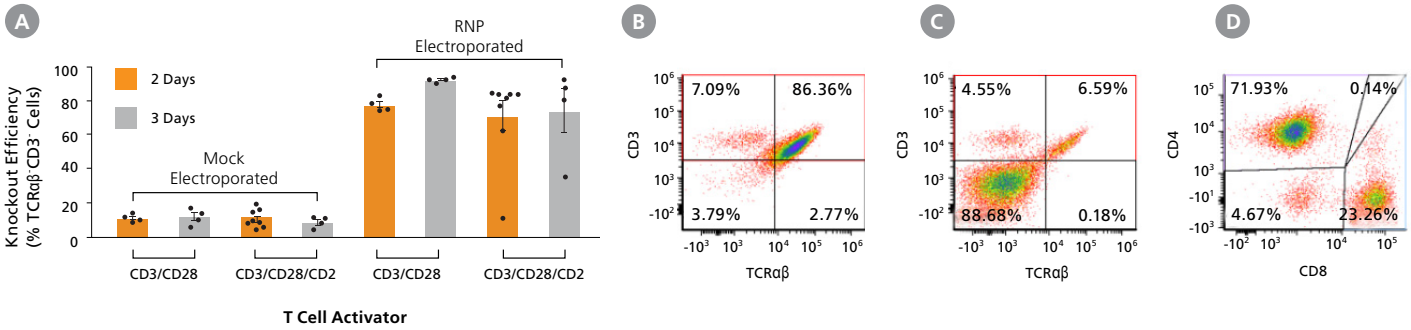


Figure 4. High Efficiency TRAC Knockout of Human T Cells

(A) TRAC knockout efficiency in human T cells activated with either ImmunoCult™ Human CD3/CD28 or CD3/CD28/CD2 T Cell Activator for 2 or 3 days was assessed by binding the TCRαβ and CD3 receptors with antibodies followed by flow cytometry analysis. Each data point per condition represents an individual donor; n = 4 - 8 donors. Error bars represent standard error of the mean. (B - C) Representative dot plots of TCRαβ and CD3 flow cytometry analysis from (B) mock electroporated and (C) RNP electroporated human T cells activated with ImmunoCult™ Human CD3/CD28 T Cell Activator for 3 days. (D) Representative dot plot of CD4 and CD8 flow cytometry analysis of human T cells activated with ImmunoCult™ Human CD3/CD28 T Cell Activator for 3 days.

Expand human T cells by culturing in ImmunoCult™-XF T Cell Expansion Medium

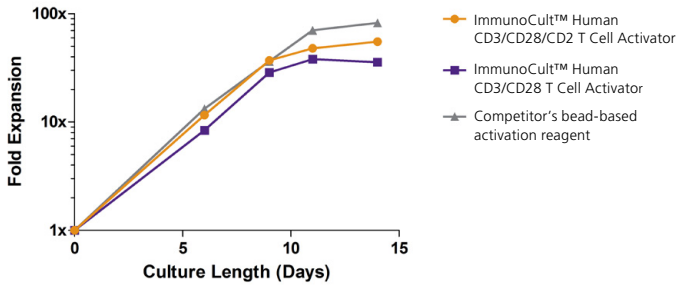


Figure 5. T Cells Show Robust Expansion When Stimulated with ImmunoCult™ Human T Cell Activators in ImmunoCult™-XF T Cell Expansion Medium

T cells were expanded over 14 days with ImmunoCult™ Human CD3/CD28 T Cell Activator, ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator or competitor's bead-based activation reagent in ImmunoCult™-XF T Cell Expansion Medium supplemented with rhIL-2. Fold expansion was determined between 0 to 14 days. (Note that T cells were not reactivated during the course of expansion.)

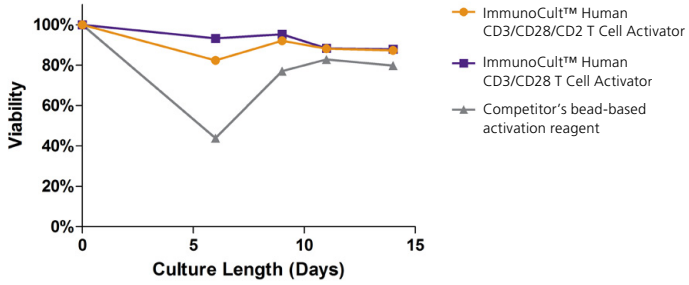


Figure 6. T Cells are Highly Viable When Stimulated with ImmunoCult™ Human T Cell Activators in ImmunoCult™-XF T Cell Expansion Medium

T cells were expanded over 14 days with ImmunoCult™ Human CD3/CD28 T Cell Activator, ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator or competitor's bead-based activation reagent in ImmunoCult™-XF T Cell Expansion Medium supplemented with rhIL-2. % viability was determined between 0 to 14 days. (Note that T cells were not reactivated during the course of expansion.)

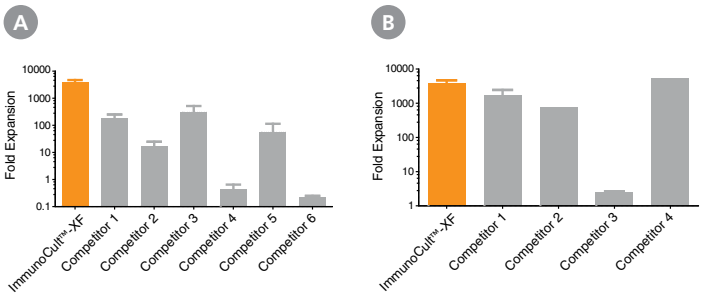


Figure 7. ImmunoCult™-XF T Cell Expansion Medium Supports Greater T Cell Expansion than Other Serum-Free and Serum-Supplemented Media

T cells were activated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator, and cultured in (A) ImmunoCult™-XF T Cell Expansion Medium or serum-free competitor media with rhIL-2 in three replicate cultures per donor, or cultured in (B) ImmunoCult™-XF T Cell Expansion Medium or serum-supplemented competitor media with rhIL-2 in three replicate cultures per donor. T cells were stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator on day 0 and every 7 to 8 days for the duration of the culture. T cells were analyzed on day 21 for fold expansion relative to the initial cell seeding density. (A) Compared to all serum-free competitor media tested, ImmunoCult™-XF T Cell Expansion Medium showed significantly higher expansion of total T cells. Competitors 1 to 6 represent serum-free competitor media. Each column with error bars represents the mean ± S.E.M. ($p < 5x10^{-13}$ for ImmunoCult™-XF T Cell Expansion Medium versus all other serum-free media, tested using the linear mixed-effect model with linear regression, n = 4 to 19 donors). (B) Compared to all serum-supplemented competitor media tested, ImmunoCult™-XF T Cell Expansion Medium showed similar or significantly higher expansion of total T cells. Competitors 1 to 4 represent serum-supplemented competitor media. Each column with error bars represents the mean ± S.E.M. ($p < 0.0006$ for ImmunoCult™-XF T Cell Expansion Medium versus all other serum-supplemented media except for competitor 4, tested using the linear mixed-effect model with linear regression, n = 1 to 19 donors).

Why Use ImmunoCult™ T Cell Activation and Expansion Reagents?

OPTIMIZED. Robust activation and rapid expansion without the use of magnetic beads.

DEFINED FORMULATION. Consistent expansion without the need to add serum.

FREEDOM TO USE. Not exclusively licensed for use in T cell therapy manufacturing.

Why Use STEMCELL's Reagents for Cell Therapy Research Applications?

CONSISTENCY. Defined formulations minimize lot-to-lot variability.

QUALITY. Extensive QC testing.

DOCUMENTATION. Traceability documentation including CoAs and CoOs help reduce time in preparing IND submissions or clinical trial applications.

CONSULTATION. Experienced global professionals to help navigate regulatory processes.

From Bench to Bedside

These products are designed for cell therapy research applications following the recommendations of USP <1043> on Ancillary Materials.

Contact us to qualify these reagents under an approved investigational new drug (IND) or clinical trial application (CTA).

Learn more at **www.stemcell.com/t-cell-therapy**