STREAMLINE YOUR IMMUNE CELL THERAPY DEVELOPMENT

Combine High-Performance ImmunoCult™-XF with ImmunoCult™ Human T Cell Activators

Activate and Expand Human T Cells for Use in T Cell Therapy Manufacturing

Streamline your cell therapy development and manufacturing by combining high-performance GMP ImmunoCult™-XF medium with GMP ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator or ImmunoCult™ Human CD3/CD28 T Cell Activator. Designed for robust and consistent T cell activation and expansion, the ImmunoCult™-XF medium is serum- and xeno-free and has no added cytokines, while the ImmunoCult™ activators are highly stable and soluble—providing complete flexibility and choice of cytokine during your workflows.

The GMP ImmunoCult™ reagents together provide optimal T cell expansion and viability without the use of magnetic beads, feeder cells, or antigens. Cultured T cells produce cytokines, including IFN-γ and TNF-α, upon restimulation. The ImmunoCult™ T cell workflow streamlines T cell therapy development by enabling process standardization and scale-up capability from discovery through to clinical and commercial manufacturing.

Why Use ImmunoCult™ for T Cell Therapy Manufacturing?

ROBUST. Expand T cells for use in cell therapy development with a medium produced under relevant GMPs.

REPRODUCIBLE. Reduce variability by expanding T cells in serum- and xeno-free culture conditions.

EFFICIENT. Achieve robust T cell expansion with high viability.

FUNCTIONAL. Obtain T cells able to produce cytokines upon restimulation.

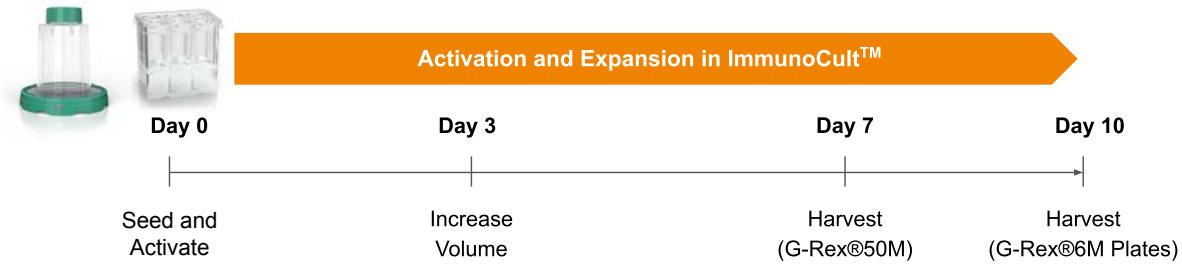
STREAMLINED. Activate T cells bead-free by combining ImmunoCult™-XF with ImmunoCult™ Human T Cell Activators from preclinical development through to commercial manufacturing.

GMP ImmunoCult™ Products

Product	Catalog #	Size
ImmunoCult™-XF	100-0956	500 mL
ImmunoCult™ Human CD3/CD28 T Cell Activator	100-0784	10 mL
ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator	100-0785	10 mL

T Cell Manufacturing in Bioreactors

ImmunoCult™ reagents support robust expansion of functional T cells using common bioreactors. For the following protocols, human pan T cells were isolated from fresh leukopaks (Catalog #70500) from healthy donors using EasySep™ Human T Cell Isolation Kit (Catalog #17951) and cryopreserved in CryoStor® CS10 (Catalog #100-1061). On the day of the experiment, cryopreserved T cells were thawed, washed, and resuspended in ImmunoCult™-XF (Catalog #100-0956) supplemented with 180 IU/mL Human Recombinant IL-2, ACF (rhIL-2; Catalog #78145).



	G-Rex®6M Plate	G-Rex® 50M Bottle
Surface Area	10 cm²	50 cm²
Volume on Day 0	10 mL	50 mL
Final Volume on Day 3	100 mL	500 mL

Figure 1. Culture Protocol for T Cell Activation and Expansion in G-Rex®6M Well Plates and G-Rex®50M Open System

Isolated T cells (fresh or frozen) were seeded at 1 - 2 x 10^6 cells/cm² in a total volume of 10 mL (G-Rex®6M well plate) or 50 mL (G-Rex®50M system) and activated with either 6.25 or 25 µL/mL of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #100-0785). On Day 3, G-Rex® cultures were topped up with fresh ImmunoCult™-XF (Catalog #100-0956) + Human Recombinant IL-2, ACF (rhIL-2; Catalog #78145) to a maximum volume of 100 mL (G-Rex®6M well plates) or 500 mL (G-Rex®50M system), as shown in the table above. No culture manipulation was performed until harvest on Day 7 if using the G-Rex®50M system or Day 10 for the G-Rex®6M well plate.

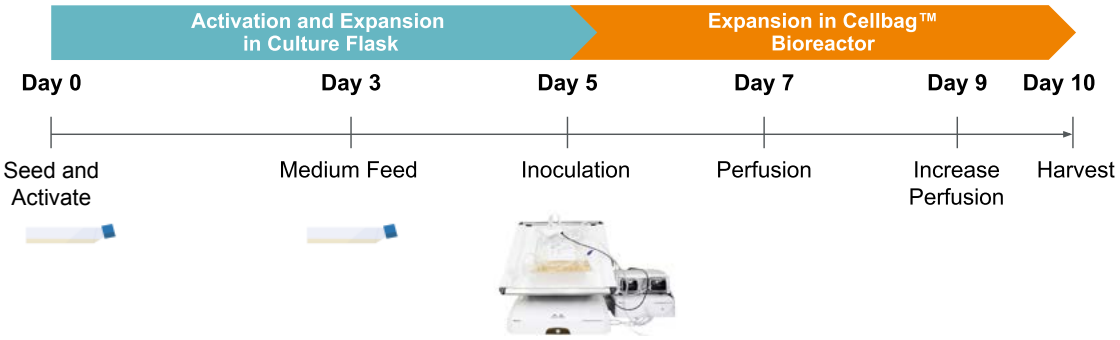


Figure 2. Culture Protocol for Expansion of T Cells in the Xuri™ Cell Expansion System W25

5 x 10^7 T cells were seeded in a T-175 tissue culture flask containing 50 mL of medium and activated with 25 µL/mL of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #100-0785). On Day 3, the cell density was adjusted to 1 - 2.5 x 10^5 cells/mL by adding fresh ImmunoCult™-XF (Catalog #100-0956) + Human Recombinant IL-2, ACF (rhIL-2; Catalog #78145). On Day 5, cell expansion on the Xuri™ platform was initiated by inoculating 3 x 10^8 T cells in 1 L of culture medium into a Xuri™ 2L Cellbag™ Bioreactor. Perfusion was initiated at 0.5 L/day on Day 7 and increased to 1 L/day on Day 9. The culture was harvested on Day 10.

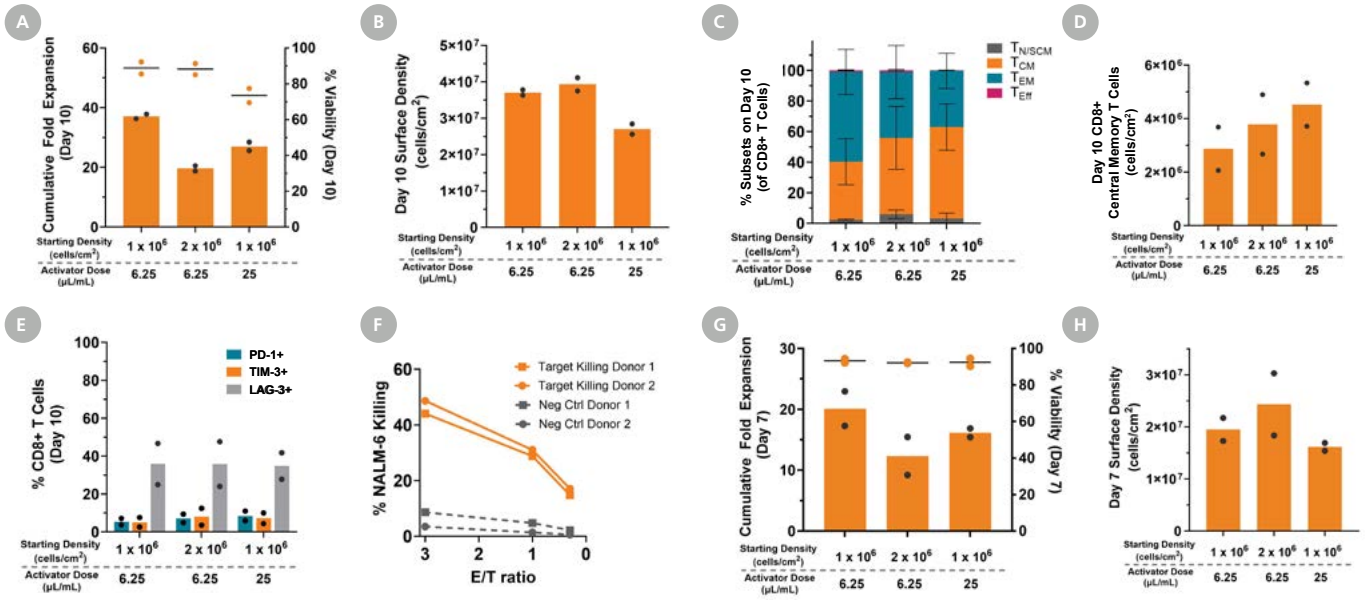


Figure 3. Flexible Activator Dosing in G-Rex® Bioreactors Enables Scalable Production of Functional T Cells

Isolated human T cells were activated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #100-0785) at 6.25 or 25 µL/mL in ImmunoCult™-XF (Catalog #100-0956) supplemented with Human Recombinant IL-2, ACF (rhIL-2; Catalog #78145). Cells were expanded either in a G-Rex®6M Plate for 10 days (A - F) or in a G-Rex®50M system for 7 days (G and H) following the protocol in Figure 1. (A) The composite bar and scatter graphs represent the average cumulative fold expansion and cell viability, respectively. (B) Surface cell densities were assessed on Day 10. A reduced dose of activator was able to yield ~4 x 10^7 T cells/cm^2, reaching the maximum capacity of the device. (C) The frequency of CD8+ T cell subsets was analyzed. By Day 10, the expanded T cell population comprised both central and effector memory subsets. (D) The surface density of central memory T cells (CD45RO+CCR7+) is shown. In all conditions, the number of central memory T cells exceeded 2 × 10^6 cells/cm^2, corresponding to a total of approximately 2 x 10^7 cells per well. (E) The frequency of LAG-3 expression among CD8+ T cells ranged from 20 - 50%, whereas TIM-3 and PD-1 expression levels were consistently below 10%. (F) The expanded T cells exhibited a robust cytotoxic capacity when co-cultured with NALM-6 tumor cells. (G) Cumulative fold expansion, cell viability, and (H) surface cell density were evaluated following a 7-day short-term T cell expansion using the G-Rex®50M system. Yields of more than 2 x 10^7 cells/cm^2 (> 1 × 10^9 cells per device) were achieved with the lower activator dose. Data represented as the mean from two independent donors, with each dot corresponding to an individual donor.

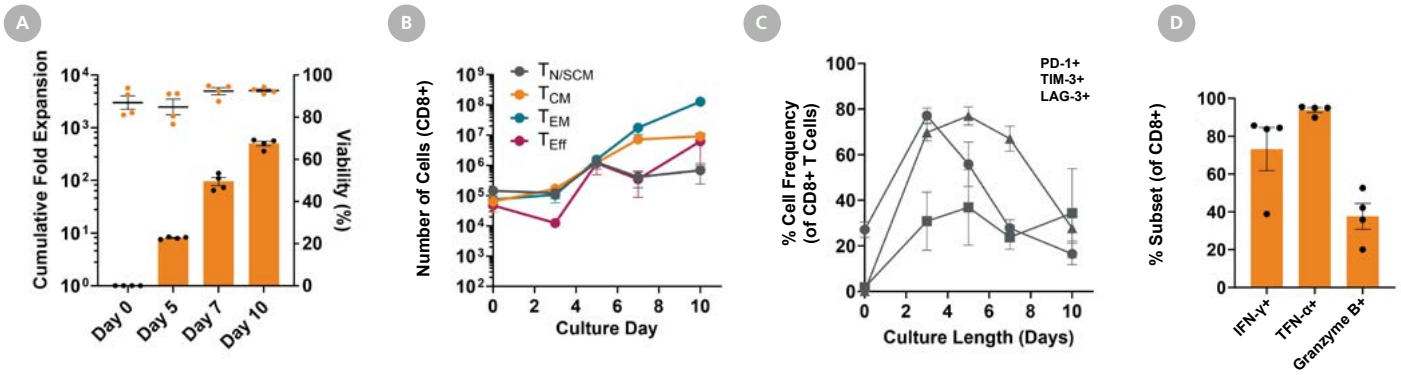


Figure 4. T Cell Expansion in the Xuri™ Cell Expansion System W25 Generates Highly-Viable and Functional Cells Capable of Producing Cytokines and Effector Molecules

T cells were activated with 25 µL/mL of ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator (Catalog #100-0785) in ImmunoCult™-XF (Catalog #100-0956) supplemented with Human Recombinant IL-2, ACF (rhIL-2; Catalog #78145). Expansion was carried out in 2 L Cellbag™ bioreactors on the Xuri™ platform, following the protocol outlined in Figure 2. (A) Cumulative fold expansion (bars) and viability (scatter graphs) over a 10-day culture period. Approximately 92.7% cell viability and a total fold expansion of 503 ± 51 were achieved by Day 10. (B) The number of T cell subtypes in CD8+ populations are shown. Cell numbers were calculated based on 1 x 10^6 starting T cells. The number of central memory T cells peaks on Day 7 and remains constant until Day 10. (C) The frequency of T cells expressing PD-1, TIM-3, and LAG-3 increased by Day 3 and subsequently decreased from Day 3 to Day 10. (D) The frequencies of expanded T cells expressing IFN-γ and TNF-α upon stimulation with PMA and ionomycin are 73.2 ± 11.4% and 93.8 ± 1.3%, in the CD8+ population. Additionally, the frequency of expanded CD8+ T cells producing granzyme B without further stimulation is 37.7 ± 6.8%. Data represented as mean ± SEM (n = 3 - 5).

Support Expansion Across Immune Cell Types

ImmunoCult™-XF supports robust immune cell expansion under defined, serum- and xeno-free conditions. In addition to T cells, ImmunoCult™-XF enables high-viability expansion of natural killer (NK) cells, extending its utility across cell therapy workflows.

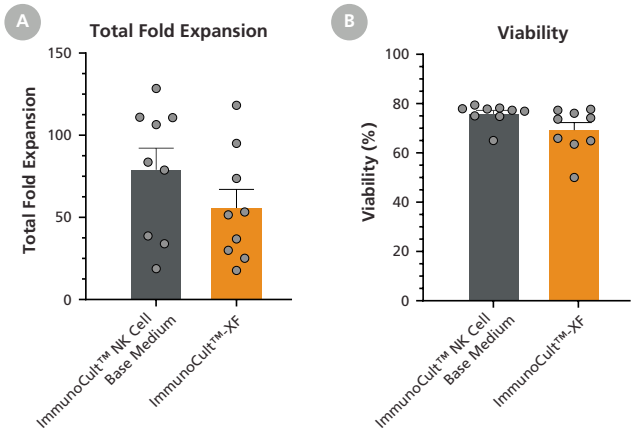


Figure 5. ImmunoCult™-XF Supports Natural Killer Cell Expansion

Natural killer (NK) cells were isolated from human peripheral blood samples using EasySep™ Human NK Cell Isolation Kit (Catalog #17955), then cultured in media supplemented with ImmunoCult™ NK Cell Expansion Supplement (Catalog #110-0715) for a total of 14 days. Cells were stimulated with ImmunoCult™ NK Cell Expansion Coating Material (Catalog #100-0714) on Days 0, 7, and 10 of culture. The medium used was either ImmunoCult™ NK Cell Base Medium (Catalog #100-0712) or ImmunoCult™-XF (Catalog #100-0956). (A) NK cells undergo robust expansion over 14 days of expansion with ImmunoCult™ NK Cell Base Medium or ImmunoCult™-XF. (B) NK cells remain highly viable after 14 days of expansion in ImmunoCult™ NK Cell Base Medium or ImmunoCult™-XF. Each column with error bars represents the mean ± SEM across three individual donors tested across three different manufactured lots of media.

Taking Your Research to the Clinic?

STEMCELL's Services for Cell Therapy program has a team of experts who can help support your regulatory filing by providing custom solutions such as quality documentation, additional product testing, and customized product manufacturing. To learn more about how we can support your preclinical and clinical research needs, visit us at **www.stemcell.com/cell-therapy-services**

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