Activation and Expansion of Human T Cells With Immobilized CD3 and Soluble CD28 Antibodies

In a G-Rex® Bioreactor

Introduction

Adoptive T cell therapy is a rapidly growing segment of cell and gene therapy. This protocol outlines how to activate and expand purified human T cells using immobilized CD3 and soluble CD28 antibodies, all within a G-Rex bioreactor. T cells are stimulated with GMP CD3 and CD28 antibodies for 2 days followed by 7 days of expansion in Bio-Techne's xeno-free GMP T cell media. Robust CD3+ T cell expansion and high cell viabilities are achieved in the G-Rex.

Abbreviations:

BSC: Biological Safety Cabinet

GMP: Good Manufacturing Practice

PBMCs: Peripheral Blood Mononuclear Cells

TABLE // 01

Materials Required

Material	Catalog Number
GMP T Cell Media	CCM038-GMP
Human IL-7 *	BT-007-GMP / BT-007-AFL
Human IL-15 *	BT-015-GMP / BT-015-AFL
Human CD3 GMP Antibody	MAB11411-GMP
Human CD28 GMP Antibody	MAB11412-GMP
G-Rex® 6 Well Plate M-Series	80240M
Human AB Serum	Multiple vendors
RectroNectin	
15 and 50 mL Centrifuge Tubes	
1X PBS	
Cell Counter	

*Note that IL-7 and IL-15 are available Animal-Free and GMP in lyophilized or liquid formulations

General Guidelines

- When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed.
- Maintain sterile technique by performing work in a BSC, wearing gloves, and using nuclease-free reagents and sterile pipette filter tips.
- All reagents should be stored according to the manufacturer's recommendations.
- If using the recommended Bio-Techne media and G-Rex bioreactor, cells will be confluent after 9 days of culture. If using a different media or reaction vessel, follow manufacturer's protocol to determine optimal culture time.

TABLE // 02

Timeline

Vessel Coating	Activation	Transposition	Expansion
Day -1 • Coat vessel with RetroNectin and CD3 antibody (overnight)	**Day 0** • Prepare media • Isolate/thaw T cells • Activate T Cells • Incubate (2 days)	**Day 2** • Prepare media to dilute the culture • Mix and count cells • Incubate (7 days)	**Day 6 (Optional)** • Check cell growth **Day 9** • Harvest cells for desired application

Protocol

Vessel Preparation

Day -1 of Total Culture Time

Immobilization of CD3 Antibody to the Vessel Surface

1. Dilute CD3 antibody and RetroNectin in sterile 1X PBS in a 50 mL conical tube.
   a. Recommended final concentration of CD3 antibody is 1 µg/mL and RetroNectin is 40 µg/mL.
   b. These concentrations can be optimized for the end-user's workflow.
2. Add 2 mL of diluted CD3 antibody and RetroNectin to each well of G-Rex.
   a. For different size G-Rex bioreactors, add 0.2 mL of diluted CD3 antibody and RetroNectin per cm^2 of G-Rex well.
3. Incubate G-Rex bioreactor overnight at 4 °C.

Activiation

Day 0 of Total Culture Time

Prepare Media and Vessel

1. Transfer G-Rex bioreactor from 4 °C to room temperature to acclimate.

2. Mix and sterile filter complete media.
   a. Human GMP T Cell Media
   b. 5% Human AB Serum
   c. 10 ng/mL IL-7
   d. 10 ng/mL IL-15

3. Remove diluted CD3 antibody and RetroNectin from G-Rex bioreactor.

4. Wash G-Rex bioreactor with sterile 1X PBS.

5. Add at least 0.2 mL sterile 1X PBS per cm^2 of G-Rex well to prevent coated vessel surface from drying out.
   a. Remove 1X PBS prior to adding T cells.

Prepare Purified T Cells

1. Either:
   a. Isolate T cells from human PBMCs following desired protocol or
   b. Thaw purified T cells and wash with complete media.

2. Aspirate supernatant.

3. Resuspend cell pellet in complete media.

4. Count resuspended cells.

Activate T Cells

1. Seed 5 x 10^6 purified T cells per well in G-Rex bioreactor.
   a. Refer to the G-Rex Plating Reference below for seeding cell number of other G-Rex vessel sizes.

2. Add CD28 antibody to final concentration of 1 µg/mL.
   a. This concentration can be optimized for the end-user's workflow.

3. Fill G-Rex bioreactor to activation volume of 10 mL with pre-warmed complete media (1 mL/cm^2).
   a. Refer to G-Rex Plating Reference to determine activation volume for different G-Rex bioreactor sizes.

4. Transfer G-Rex bioreactor to a humidified incubator (37 °C, 5% CO_2) and incubate for 2 days.

Increase Culture Volume

Day 2 of Total Culture Time

Prepare Media

1. Pre-warm desired volume of complete media needed to dilute cell culture.

Fill G-Rex Bioreactor

1. Gently mix the activated cell complexes to break them apart, approximately 10-15 times.
   a. If desired, cell counts can be performed at this step.
   b. Note: We have observed disrupting cell complexes at this time improves cell expansion.

2. Fill G-Rex bioreactor to maximum fill volume of 40 mL with pre-warmed complete media.
   a. Refer to the G-Rex Plating Reference for fill volumes of different G-Rex vessel sizes.

3. Place G-Rex bioreactor into humidified incubator at 37 °C, 5% CO_2.

Expansion

Optional: Day 6 of Total Culture Time

Prepare Media and Perform ½ Media Exchange

1. Pre-warm volume of compete media needed to complete a ½ media exchange in a G-Rex 6 well bioreactor.
   a. Note: If using G-Rex 6M, 100M, or 500M, cells and media do not need handling and can be left untouched until day 9 of total culture time.
2. Transfer G-Rex bioreactor to BSC.
3. Carefully volume reduce wells of G-Rex to ~2 mL/cm^2.
4. Add pre-warmed complete media to wells to fill G-Rex bioreactor.
5. Proceed to check cell growth, or place G-Rex bioreactor into incubator at 37 °C, 5% CO_2.

Check Cell Growth

1. Cell counts and other analysis can be performed at this time.
2. Mix cells and sample each well for cell counts and desired analysis.
3. Place G-Rex bioreactor into incubator at 37 °C, 5% CO2 until day 9 of total culture.

Day 9 of Total Culture Time

Harvest Cells

1. Reduce the volume of the cell culture media in the G-Rex bioreactor to ~1-2 mL/cm^2.
2. Mix the cells and sample each well for final cell counts and desired flow cytometry applications for phenotype characterization.
3. Cryopreserve remaining cells or use directly for desired applications.

TABLE // 03

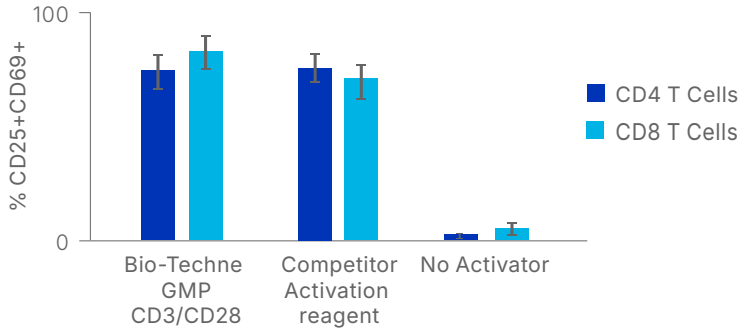
G-Rex Plating Reference for Purified T Cells

G-Rex Format	cm^2	Cell # for Activation	Activation Capacity	Maximum Capacity	Confluency
G-Rex 24 Well Plate	2 cm^2	1 x 10^6	2 mL	8 mL	60-80 x 10^6
G-Rex 6 Well Plate	10 cm^2	5 x 10^6	10 mL	40 mL	350-400 x 10^6
G-Rex 6M Well Pate	10 cm^2	5 x 10^6	10 mL	100 mL	350-400 x 10^6
G-Rex 100M	100 cm^2	50 x 10^6	100 mL	1,000 mL	3.5-4 x 10^9
G-Rex 500M	500 cm^2	250 x 10^6	500 mL	5,000 mL	15-20 x 10^9

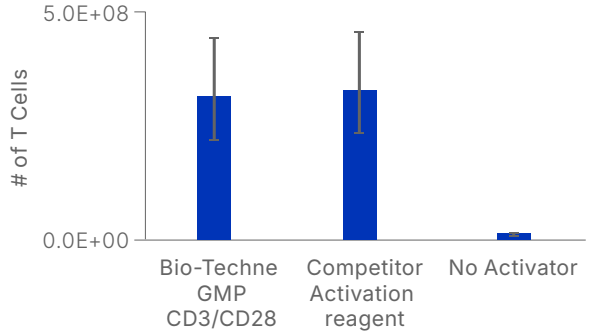
Representative Data

FIGURE // 01

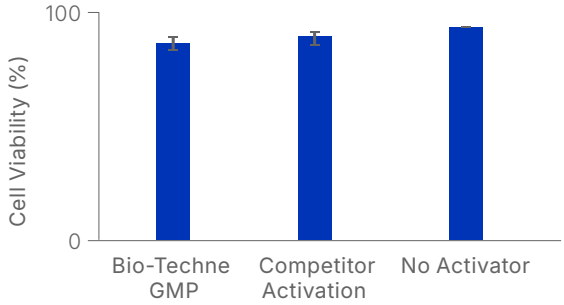
A. Expression of Activation Markers on Day 2



B. T Cell Count at Harvest



C. Cell Viability at Harvest



Immobilized CD3 and soluble CD28 GMP antibodies promote T cell expansion as well as competitor activation reagents. Purified human T cells from three independent donors were activated with GMP CD3 (Catalog # MAB11411-GMP) and CD28 (Catalog # MAB11412-GMP) antibodies for 2 days and expanded in GMP Human T Cell Media (Catalog # CCM038-GMP) supplemented with 5% human AB serum and 10 ng/mL of GMP IL-7 (Catalog # BT-007-GMP) and IL-15 (Catalog # BT-015-GMP) for 7 days in a 6M G-Rex bioreactor. After 48 hours of stimulation, levels of activation markers were analyzed by flow. Upregulation of CD25 and CD69 markers in CD4+ and CD8+ T cells indicate that cells were successfully activated with immobilized CD3 and soluble CD28 antibodies after two days of stimulation **(A)**. Total cell numbers **(B)** and cell viabilities **(C)** were measured on day 9 of culture. Data is average ± SD of n=3 donors.