

# Activation and Expansion of Human T Cells

## With Bead-Bound GMP CD3 and CD28 Antibodies

### 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 R&D Systems CD3 and CD28 antibodies conjugated to beads. 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® bioreactor.

#### ABBREVIATIONS

BSC: Biological Safety Cabinet  
 GMP: Good Manufacturing Practices  
 PBMCs: Peripheral Blood Mononuclear Cells

### Materials Required

Material	Catalog Number
Human 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 Antibody	MAB11411-GMP
Human CD28 Antibody	MAB11412-GMP
G-Rex® 6 Well Plate M-Series	80240M
Magnetic Beads Coupled to Streptavidin	
Human AB Serum	
15 and 50 mL Centrifuge Tubes	Multiple vendors
Biotin Labeling Kit	
Cell Counter	

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

### Timeline

Activation	Dilution	Expansion
<b>Day 0</b> <ul style="list-style-type: none"> <li>Prepare media</li> <li>Isolate/thaw T cells</li> <li>Stimulate with CD3/CD28 activator</li> <li>Incubate (2 days)</li> </ul>	<b>Day 2</b> <ul style="list-style-type: none"> <li>Prepare media to dilute the culture</li> <li>Mix and count cells</li> <li>Fill G-Rex bioreactor</li> <li>Incubate (7 days)</li> </ul>	<b>Day 6 (optional)</b> <ul style="list-style-type: none"> <li>Media exchange</li> <li>Check cell growth</li> </ul> <b>Day 9</b> <ul style="list-style-type: none"> <li>Harvest cells for desired application</li> </ul>

## General Guidelines

- When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed.
- Maintain sterile technique, wearing gloves, 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.

## Protocol

### ANTIBODY PREPARATION

#### PRIOR TO CELL CULTURE

#### Biotinylation and Immobilization of the Antibodies on Magnetic Beads

1. Conjugate CD3 and CD28 antibodies to biotin.
  - a. Follow manufacturer instructions from desired kit.
2. Immobilize biotin-conjugated CD3 and CD28 antibodies to magnetic beads coupled to streptavidin.
  - a. Following manufacturer instructions from kits of choice.
  - b. NOTE: For the representative data at the end of this protocol, biotinylated CD3 and CD28 antibodies were immobilized on Dynabeads M-270 Streptavidin (Catalog # 65305) at a 1:3 CD3/CD28 ratio.
3. Store at 4 °C until use.

### ACTIVATION

#### DAY 0 OF TOTAL CULTURE TIME

#### Prepare Media

1. 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
2. NOTE: Complete media can be stored at 2 - 8 °C, protected from light for 2 weeks.

#### Thaw T Cells

3. Either:
  - a. Isolate T cells from human PBMCs following desired protocol or
  - b. Thaw purified T cells and wash with complete media.
4. Aspirate supernatant and resuspend cell pellet in complete media.
5. Count resuspended cells.
6. Seed  $0.5 \times 10^6$  cells/cm<sup>2</sup> in 1 mL/cm<sup>2</sup> of complete media per well in G-Rex plate.
  - a. NOTE: When adding 1 mL/cm<sup>2</sup> complete media, take into account the volume of activation beads that will be added to the bioreactor.
  - b. Refer to the G-Rex Plating Reference for seeding cell number and vessel sizes.

### **Wash CD3/CD28 Beads Prior to Use**

7. Take the desired volume of pre-made CD3/CD28 antibodies conjugated to magnetic beads and place in an appropriately sized tube.
  - a. NOTE: 3 beads per cell are needed for activation.
  - b. EXAMPLE: Prepare beads to activate  $5 \times 10^6$  cells.
    - I. CD3/CD28 antibody conjugated beads are at  $3 \times 10^7$  beads per mL.
    - II.  $15 \times 10^6$  beads are needed for  $5 \times 10^6$  cells.
    - III. Remove 0.5 mL of CD3/CD28 antibody conjugated beads from stock.
8. Place the tube on a magnet for 1 minute.
9. Without removing the tube from the magnet, carefully remove the supernatant.
10. Remove the tube from the magnet and resuspend in the same volume of complete media as the initial volume taken from the stock.

### **Stimulate With CD3/CD28 Activator**

11. Add CD3 and CD28 activation reagent to cells.
12. Gently pipet mix to evenly distribute the activators in the culture.
13. Transfer G-Rex bioreactor to a humidified incubator (37 °C, 5% CO<sub>2</sub>) and incubate for 2 days.

## **DILUTION**

DAY 2 OF TOTAL CULTURE TIME

### **Prepare Media**

1. Pre-warm volume of complete media needed to fill the G-Rex bioreactor.

### **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 to 4 mL/cm<sup>2</sup> per well in the non-M series or 10 mL/cm<sup>2</sup> per well in the M series with pre-warmed complete media.
  - a. Refer to the G-Rex Plating Reference table for fill volumes of other vessel sizes.
3. Place G-Rex bioreactor into incubator at 37 °C, 5% CO<sub>2</sub>.

## EXPANSION

### OPTIONAL: DAY 6 OF TOTAL CULTURE TIME

#### Prepare Media and Perform ½ Media Exchange

1. Pre-warm volume of complete media needed to complete 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<sup>2</sup>.
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<sub>2</sub>.

#### Check Cell Growth (Optional)

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% CO<sub>2</sub> until day 9 of total culture.

### DAY 9 OF TOTAL CULTURE TIME

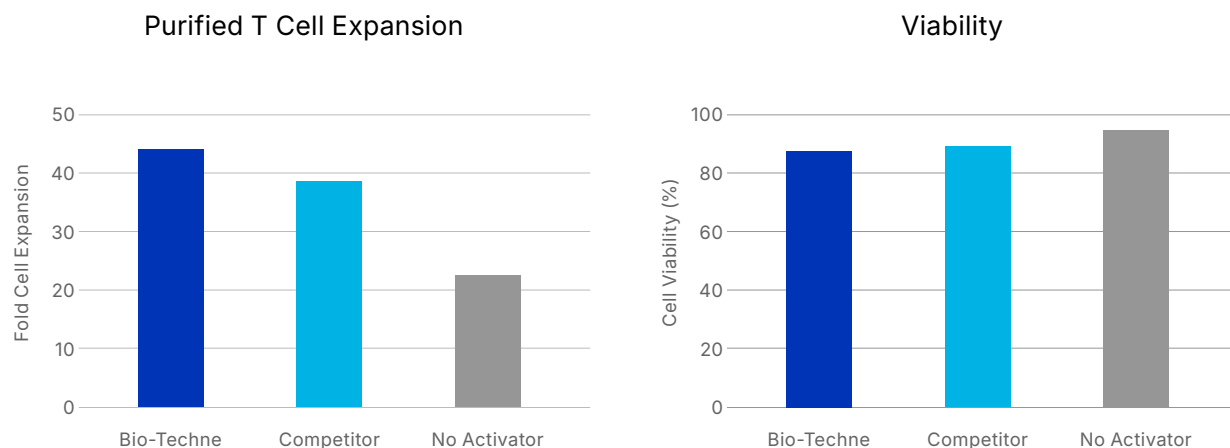
#### Harvest Cells

1. Reduce the volume of the cell culture in G-Rex 6-well to ~2 mL/cm<sup>2</sup> (about 1/2 of the volume capacity).
2. Mix the cells and sample each well for final cell counts and for desired flow cytometry applications for phenotype characterization.
  - a. NOTE: It is recommended to remove the activation beads prior to cell phenotyping, flow cytometry, functional assays and cryopreservation.
3. Cryopreserve remaining cells or use directly for desired applications.

## G-Rex Plating Reference for Purified T Cells

G-Rex Format	cm <sup>2</sup>	Cell # for Activation	Activation Volume	Volume Post Activation	Confluency
G-Rex 24 Well Plate	2 cm <sup>2</sup>	1 x 10 <sup>6</sup>	2 mL	8 mL	60-80 x 10 <sup>6</sup>
G-Rex 6 Well Plate	10 cm <sup>2</sup>	5 x 10 <sup>6</sup>	10 mL	40 mL	350-400 x 10 <sup>6</sup>
G-Rex 6M Well Plate	10 cm <sup>2</sup>	5 x 10 <sup>6</sup>	10 mL	100 mL	350-400 x 10 <sup>6</sup>
G-Rex 100M	100 cm <sup>2</sup>	50 x 10 <sup>6</sup>	100 mL	1,000 mL	3.5-4 x 10 <sup>9</sup>
G-Rex 500M	500 cm <sup>2</sup>	250 x 10 <sup>6</sup>	500 mL	5,000 mL	15-20 x 10 <sup>9</sup>

## Representative Data: CD3/CD28 Antibodies Bound to Beads



### BIO-TECHNE'S CD3 AND CD28 ANTIBODIES BOUND TO MAGNETIC BEADS PROMOTE EXPANSION OF PURIFIED T CELLS.

Purified T cells from two independent donors were thawed and activated using streptavidin magnetic beads conjugated with CD3 (Catalog # [MAB11411-GMP](#)) and CD28 (Catalog # [MAB11412-GMP](#)) antibodies. The cells were cultured for 9 days in 6 well G-Rex bioreactors (Catalog # [80240M](#)) using GMP T Cell Media (Catalog # [CCM038-GMP](#)) supplemented with 10 ng/mL liquid GMP IL-7 (Catalog # [BT-007-GMP](#)) and IL-15 cytokines (Catalog # [BT-015-GMP](#)). Bars represent the average of two donors.

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