# Human T Cell Depletion Kit (Product number: 13510-231)

Control Number UG13510231A01

## BACS™ Technology User Guide

## Description

Akadeum Human T Cell Depletion Kit, depletes endogenous human CD3+ T cells from various samples, such as PBMC (human peripheral blood mononuclear cells) and cell cultures. This kit serves fast and efficient isolation of human CD3+ T cells without columns and magnets for downstream human CD3+ T cell applications, for example, human CD3+ T cell cultures, as well as depletion of human CD3+ T cells from the sample, particularly useful for allogenic CAR-T (chimeric antigen receptor T cells) cell therapy of purifying therapeutic effective, engineered CAR-T cells. Akadeum's T Cell Depletion microbubble targets CD3+ cells with Akadeum's Human T Cell Depletion antibody cocktail recognizing the human T cell receptor-CD3 complex, CD3+ cells are labeled with antibodies and T cell depletion microbubbles, isolated by buoyance with simple centrifugation. Unlabeled non-CD3+ cells are pelleted and recovered by removal of microbubble-bound CD3+ cells, ready to use for downstream applications, such as cell culture, flow cytometric characterization, molecular assays or cell storage.

## Kit Components

- Human T Cell Depletion Antibody Cocktail, 1mL
- Human T Cell Depletion Microbubbles, 10mL

***Kit capacity: 1 x $10^9$ total cells***

## Notes Before Starting

- Recommended supplies:
  - Vortex mixer
  - Mixer with end-over-end rotation set at 20 rpm
  - Centrifuge with swing bucket rotor
  - Vacuum aspirator
- Buffer: Phosphate-buffered saline (PBS, $Ca^{2+}$ and $Mg^{2+}$ free) supplemented with 0.5% bovine serum albumin (BSA, biotin free), pH 7.2 or preferred medium supplemented with 2% human serum (HS).
- Akadeum Human T Cell Depletion Kit has been optimized for nucleated cells, for example, PBMCs, with 30% - 75% starting CD3+ cell content. For sample below 30% or above 75% CD3+ cell content, optional second round of depletion could be performed, yield might decrease 10% - 20% for each additional depletion.
- Prepare cells to 333 x $10^6$ cells/mL in recommended buffer prior to depletion process.
- The recommended amounts of T Cell Depletion Antibody Cocktail and T Cell Depletion Microbubbles is 100 µL and 1 mL, respectively, per 100 x $10^6$ cells. This kit is designed for starting sample containing 10 x $10^6$ – 1000 x $10^6$ total cells in 1.7 mL, 5 mL or 50 mL centrifuge tubes, for samples greater than this range, please scale up accordingly and using appropriate vessels.

## Steps:

This guide is described on 100 x $10^6$ cells (300 µL) as starting sample, however, the process is scalable from 10 x $10^6$ – 1000 x $10^6$ cells (30 µL – 3 mL). Please reference Table 1 for volume and vessel considerations.

1. Transfer 300 µL cells to a 5 mL tube and add 100 µL T cell Depletion Antibody Cocktail.
2. Gently mix by tapping the tube or brief 1 – 2 seconds vortex the sample tube to ensure even mixture. Gentle up and down pipetting is also sufficient, be careful to avoid air bubbles (foaming) during pipetting.
3. Incubate the sample tube at room temperature for 10 minutes.
4. Resuspend T Cell Depletion Microbubbles by rolling the vial several times between hands, followed by inverting multiple times to reach a homogeneous suspension and making sure T Cell Depletion Microbubbles are thoroughly resuspended immediately prior to addition to sample.
5. Add 1 mL of T cell Depletion Microbubbles to the labeled cell sample from Step 4.3. at the end of 10 minutes incubation and 2.6 mL buffer to achieve a final volume of 4 mL (approximately 80% volume of the tube capacity).
6. Incubate the sample tube with 20 rpm end-over-end rotation for 15 minutes at room temperature.
7. Retrieve the tube from rotator and centrifuge for 400g, 5 min, use of a swing bucket rotor is recommended to facilitate microbubble aspiration.
8. Carefully retrieve the sample tube from centrifuge with minimal disturbance of T Cell Depletion Microbubble layer on the top of the tube. Use a vacuum aspirator to carefully remove the white microbubble layer and supernatant, be careful not to disturb the cell pellet.
9. Resuspend cell pellet with small amount desired cell medium and transfer to a new tube for further use in downstream applications.remove the white microbubble layer and supernatant, be careful not to disturb the cell pellet.

## Table 1. Examples of volume and vessel consideration for Human T cell depletion

| Sample Size | Tube Size | Cell Suspension | T Cell Depletion cocktail | T Cell depletion Microbubbles | Buffer |
|---|---|---|---|---|---|
| 10 x $10^6$ cells | 1.7 mL | 30 µL | 30 µL | 100 µL | 1.2 mL |
| 100 x $10^6$ cells | 5mL | 300 µL | 300 µL | 1 mL | 2.6 mL |
| 1000 x $10^6$ cells | 50mL | 3 mL | 3 mL | 10 mL | 26 mL |

## Safety Information

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

For information regarding hazards and safe handling practices, please consult the Safety Data Sheet.

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