

# Human T Cell Depletion Kit - CR

13510-232CR Document Number UG13510232CRA02

#### Kit Contents:



REF

50 mL Human T Cell Depletion Microbubbles -CR in storage buffer

5 mL Human T Cell Depletion Antibody Cocktail - CR in sterile Separation Buffer.

# Expiration dates are indicated on the labels for each individual component.

#### Storage



This product is shipped refrigerated and must be stored between +2 °C and +8 °C immediately upon receipt. Do not freeze.

# **Product Description**

The Akadeum Human T Cell Depletion Kit depletes endogenous human CD3+ T cells from various samples such as cell cultures. This kit serves as a method to rapidly and efficiently deplete human CD3+ T cells without columns and magnets, which is particularly useful for allogeneic CAR-T (chimeric antigen receptor T cells) manufacturing processes. Akadeum's Human T Cell Depletion Antibody Cocktail recognizes the human Ť cell receptor-ČD3 complex, labeling CD3+ cells and leading to T cell depletion through binding of microbubbles and separation through buoyancy with simple centrifugation or gravity. Unlabeled non-CD3+ cells can be recovered by removal of microbubble-bound CD3+ cells. Non-CD3+ cells are untouched and ready to use for downstream applications, such as cell culture, flow cytometric characterization, molecular assays, or cell storage.

# Intentions for Use

This kit is designed to deplete CD3+ T cells from a maximum of 5 x 10<sup>9</sup> starting cells.

The components of the Human T Cell Depletion Kit – CR are intended for the *ex vivo* depletion of human T cells from various samples for cell-based clinical research. They are not intended for human *in vivo* uses.

# **Quality Statement**

Akadeum CR products are manufactured according to cGMP at Akadeum Life Sciences®, Ann Arbor, MI, under a quality management system in compliance with 21 CFR 820, 210, 211, and 11. They are developed following ISO 20399 and USP < 1043> recommendations on ancillary materials and

tested according to ISO

All components tested for endotoxins as per USP <85> Bacterial Endotoxins.

10993.

Sterile as per USP <71> Sterility Tests.

Antibody cocktail manufactured asepctically. Buffer processed with 0.2 µm filtration.

**STERILE R** Microbubbles sterilized with in-process electron beam and then filled aseptically.

# Safety Information

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

# **Related Products**

Warnings



Do not use after the use-by date listed on the product label.



Do not use product if package is damaged.

#### Handling Guidelines

- When working with human blood products, including; cells, serum, and plasma, follow universal precautions.
- Proper Personal Protective Equipment (PPE) including lab coats, gloves, and eye protection are recommended when working with human tissues.
- Open solution transfers like spike connections and pipetting must be performed under a class 100 biological safety cabinet using aseptic technique.
- Human blood products must be treated as a potential source of HIV, HBV, and other bloodborne pathogens.
- Materials contaminated with blood products should be disposed of in labeled biohazard containers or decontaminated by site approved decontamination methods.

# Additional Supplies Required

- 20 rpm end-over-end tube rotator for mixing
- Centrifuge (swinging bucket rotor strongly recommended)
- Vacuum aspirator
- Sterile 50 mL tubes
- Buffer of choice

# **Limited Warranty**

Akadeum Life Sciences® warrants their products as set forth in their General Terms and Conditions. Questions and requests can be sent to info@ akadeum.com.

Catalog Number	Product
13210-221GMP	Human T Cell Leukopak Isolation Kit - GMP
13310-224GMP800	Human T Cell Selection, Activation, and Expansion Kit - GMP
13310-224CR800	Human T Cell Selection, Activation, and Expansion Kit - CR
13510-232GMP	Human T Cell Depletion Kit - GMP (5 Billion Cells)

# GMP Clinical Ready

### **Before You Begin**

- This user guide is designed for isolations using 1 x 10° cells (3 mL) as starting sample, however, the process is scalable from 10 x 10<sup>6</sup> – 5 x 10° cells. For alternative starting numbers, please contact techsupport@akadeum.com.
- For maintenance of sterility, cell isolation should be conducted in a biosafety cabinet using aseptic technique.

#### Instructions for Use

#### Label cells to be depleted

- 1. Prepare cells to 333 x 10<sup>6</sup> cells/mL in buffer of choice prior to depletion process.
- 2. Transfer 3 mL cells to a fresh, sterile 50 mL tube and add 1 mL T Cell Depletion Antibody Cocktail.
- 3. Gently vortex the sample tube for 1 2 seconds to ensure even mixture. Gentle up and down pipetting is also sufficient. Be careful to avoid air bubbles (foaming) during pipetting.
- 4. Incubate the sample tube at room temperature for 10 minutes.

#### **Bind Depletion Microbubbles**

- 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.
- 6. At the end of the 10 minute incubation from Step 4, add 10 mL of T Cell Depletion Microbubbles and 26 mL buffer to the labeled cell sample to achieve a final volume of 40 mL (approximately 80% volume of the tube capacity).
- 7. Mix using a commercial end-overend rotator at 20 rpm for 15 minutes at room temperature.

### Separate cells

- 8. Centrifuge for 5 minutes at 400g at room temperature. Use of a swing bucket rotor is strongly encouraged.
- 9. Carefully retrieve the sample tube from centrifuge with minimal disturbance of T Cell Depletion Microbubble layer. Use a vacuum aspirator to carefully remove the white microbubble layer and supernatant while being careful not to disturb the remaining cells of interest.
- 10. Resuspend cells with small amount desired cell medium and transfer to a new tube for further use in downstream applications.

#### **Glossary of Symbols:**





Do not use if package is damaged

