

# Human T Cell Selection, Activation, and Expansion Kit - GMP Grade

(Catalog Number 13310-224GMP800)

Revision Number UG13310224G8A01

#### BACS™ Microbubbles User Guide

#### Kit Contents

- ▶ 8 mL BACS<sup>™</sup> Selection, Activation, and Expansion Microbubbles - GMP in sterile storage buffer
- 0.08 mL Human T Cell Selection, Activation, and Expansion Antibody Cocktail - GMP in sterile PBS

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

#### Storage

► This product must be stored at +2 °C and +8 °C immediately upon receipt. Do not freeze.

#### **Endotoxins**

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

#### Sterility

► Sterile as per USP <71> Sterility Tests. All components manufactured and filled aseptically.

# **Product Description:**

- The Human T Cell Selection, Activation, and Expansion Kit - GMP was developed with BACS™ Microbubbles to isolate, activate, and expand T cells from peripheral blood mononuclear cell (PBMC) populations in one simple system.
- This kit is designed to isolate, activate, and expand T cells from up to 800 x 106 starting PBMCs.
- ► The components of the Human T Cell Selection, Activation, and Expansion Kit GMP are intended for the ex vivo isolation of human T cells from PBMCs for cell-based clinical research. They are not intended for human in vivo use.

# **Quality Compliance Statement**

Akadeum GMP products are manufactured according to cGMP at Akadeum Life Sciences, Ann Arbor, MI, under a quality management system in compliance with 21 CFR 820, 211, and 11.

# **Additional Supplies:**

- 20 rpm end-over-end tube rotator for mixing
- 2 Centrifuge (swiinging bucket rotor strongly recommended)
- 3 Sterile 9 inch Pasteur pipets
- 4 Sterile 5 mL tubes / 50 mL tubes
- Culture vessel of choice for expansion
- 6 Cytokine supplements, as needed
- (7) Medium of choice

# Before You Begin:

- ► This user guide has been written for PBMCs or pre-isolated T cells. For alternative starting materials, please contact techsupport@akadeum.com.
- For maintenance of sterility, cell isolation should be conducted in a biosafety cabinet using aseptic technique.

#### Instructions for Use

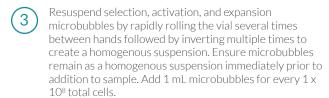
# Isolation of T cells with microbubbles using positive selection



Resuspend PBMCs or pre-isolated T cells in desired culture media (without cytokines) at approximately  $3.3 \times 10^8$  cells / mL.

Note: Both Serum-free and serum-containing media formulations are acceptable; the addition of serum may improve T cell capture as well as increase expansion kinetics. For alternative starting materials, such as platelet washed apheresis material, or for in-bag closed system workflows contact techsupport@akadeum.com.





Note: Add additional media, if needed, such that approximately 50-80% of the total vessel volume should be filled with media.





After centrifugation, the positively selected cells will be at the top of the suspension with the selection, activation, and expansion microbubbles. The remaining non-selected cells will be in a cell pellet or in suspension at the bottom of the vessel. Carefully retrieve the sample with minimal disturbance of the microbubble-cell layer. Using a sterile 9" glass pipet, insert the tip below the microbubble-cell layer and slowly descend to the bottom of the tube, manually aspirate the cell pellet and subnatant with an electronic pipette and transfer them to a new tube. Be careful not to aspirate the floating microbubble-bound cells.

Note: Retaining the subnatant and unwanted cells will allow for indirect quantification of T cell capture efficiency.

# Quantification of the positively selected T cells



Resuspend the bubble-bound cells in 1 mL of desired culture media and set it aside.



Count the cells in the subnatant using an automated cell counter or preferred cell counting technique and subtract this value from the starting cell number to determine the number of T cells captured in the bubble-cell layer.



Resuspend the microbubble-bound cells remaining in the original vessel in complete T cell medium (or other desired medium with preferred cytokine supplementation). Add approximately 1 mL media for every  $0.5\times10^6\,\mathrm{T}$  cells recovered. Akadeum recommends adding cytokine supplements for T cell culture, such as 50 U/mL IL-2, 5 ng/mL IL-7 and 5 ng/mL IL-15. Cytokine concentrations should be optimized for your particular needs.

### Seeding of T cells in cell culture medium



Distribute cells at a concentration of 0.5 x 10<sup>6</sup> T cells / mL in a desired cell culture vessel for incubation.

#### Ongoing expansion of T cells



Monitor cell growth and refresh media and cytokines every 2-3 days as needed. For well plate studies, remove half of the media from the midnatant and replace with fresh media and cytokines. For bag culture studies, add fresh media with cytokines.



When the cell density exceeds  $2 \times 10^6$  -2.5 x  $10^6$  cells/mL, transfer the cells into a larger vessel and/or dilute them to 0.5 x  $10^6$  cells/mL to allow for further expansion.

Note: Removal of the selection, activation, and expansion microbubbles from culture is not required. However, microbubbles may be removed from the culture 2 days after transduction (or a minimum of 4 days in culture).

# Glossary of Symbols







**Catalog Number** 

Temperature Limit

**Product Grade** 

# 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.



Visit us at www.akadeum.com for how-to videos, additional product information, and tech support

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