AKADEUM
LIFE SCIENCES

Human T Cell Depletion Kit - GMP Grade

REF (Catalog Number 13510-231GMP)

Revision Number UG13510231GA05

BACS™ Microbubbles User Guide

Kit Contents:

QTY

10 mL BACS™ Depletion Microbubbles - GMP in sterile storage buffer

1 mL Human T Cell Depletion Antibody Cocktail - GMP in sterile PBS

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

Storage:

This product is shipped refrigerated and 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 A

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

Product Description:

- The Human T Cell Depletion Kit - GMP was developed with BACS™ Microbubbles to deplete endogenous CD3+ T cells from various samples, such as peripheral blood lymphocytes (PBL) and cell cultures.
- The Human T Cell Depletion Antibody Cocktail recognizes CD3, a part of the T cell receptor complex using multiple, non-overlapping anti-CD3 clones for enhanced performance. CD3+ cells are labeled with antibodies, which are bound by the BACS™ Depletion Microbubbles and removed from the sample using buoyancy and simple centrifugation. Unlabeled non-CD3+ cells are recovered by removal of microbubble-bound CD3+ cells, ready to use for downstream applications.
- This kit is designed to deplete CD3+ T cells from a maximum of 1×10^9 starting cells.
- The components of the Human T Cell Depletion Kit – GMP are intended for the *ex vivo* isolation of human T cells from PBLs for cell-based clinical research. They are not intended for human *in vivo* uses.

Quality Compliance Statement:

GMP

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. They are developed following USP <1043> recommendations on ancillary materials.

Warnings:

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

Do not use product if package is damaged.

Additional Supplies:

1. 20 rpm end-over-end tube rotator for mixing
2. Centrifuge (swinging bucket rotor strongly recommended)
3. Vacuum aspirator
4. Sterile 5 mL tubes / 50 mL tubes
5. Buffer of choice. Recommended: Phosphate-buffered saline (PBS, Ca2+ and Mg2+ free) supplemented with 0.5% serum and 2 mM EDTA, pH 7.2.

Before You Begin:

- This user guide is designed for isolations using 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. For alternative starting numbers, please contact techsupport@akadeum.com.
- The recommended volumes 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 sterile 1.7 mL, 5 mL or 50 mL centrifuge tubes.
- 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 10^6 cells/mL in buffer of choice (for recommended buffer, refer to the Additional Supplies section above) prior to depletion process.
2. Transfer 300 µL cells to a fresh, sterile 5 mL tube and add 100 µL T cell Depletion Antibody Cocktail.
3. Gently mix by tapping the tube or with a brief 1 – 2 seconds vortex the sample tube to ensure even mixing. Gentle up and down pipetting is also sufficient, but be careful to avoid air bubbles (foaming) during pipetting.
4. Incubate the sample tube at room temperature for 10 minutes.

Bind Depletion Microbubbles

5. 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 1 mL of T Cell Depletion Microbubbles and 2.6 mL buffer to the labeled cell sample to achieve a final volume of 4 mL (approximately 80% volume of the tube capacity).
7. Mix using a commercial end-over-end 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:

REF	Catalog Number
QTY	Contents of packaging
GMP	Manufactured using Good Manufacturing Practices
STERILE A	Sterilized using aseptic processing techniques
	Temperature Limit
	Use-by date
	Do not use if package is damaged

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

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