



AKADEUM
LIFE SCIENCES

Human T Cell Leukopak Isolation Kit - GMP Grade

(Catalog Number 13210-221GMP)

Revision Number UG13210221GB05

BACS™ Microbubbles User Guide

Kit Contents

- ▶ 157 mL BACS™ Streptavidin Microbubbles - GMP in sterile storage buffer
- ▶ 12.5 mL Human T Cell Leukopak Biotin Antibody Cocktail - GMP in sterile PBS
- ▶ 500 mL Separation Buffer - GMP (Ca²⁺ and Mg²⁺ -free sterile PBS containing 2 mM EDTA and 0.5% biotin-free BSA)

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 Leukopak Isolation Kit - GMP was developed with BACS™ Microbubbles to isolate T cells from leukopak (apheresis) material via negative selection
- ▶ The components of the Human T Cell Leukopak Isolation Kit - GMP are intended for the *ex vivo* isolation of human T cells from leukapheresis material for cell-based clinical research. They are not intended for human *in vivo* uses.

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:

- 1 Cell washing instrument (optional)
- 2 20 RPM end-over-end rotator for Leukopak mixing
- 3 Leukopak hanger
- 4 Sterile transfer bag(s) or equivalent
- 5 Sterile tube welder
- 6 Complete cell culture media

Before You Begin:

- ▶ This user guide has been written for leukopak (apheresis) material. For alternative starting materials, please contact techsupport@akadeum.com.
- ▶ This workflow has been tested with multiple upstream and downstream systems. There may be subtle changes required to optimize each unique combination.
- ▶ This protocol is designed for starting samples containing 1×10^9 – 12.5×10^9 total cells. For samples outside of this range or for recommendations on bag sizes, please contact techsupport@akadeum.com.

Instructions for Use

Bind Antibody Cocktail from the Human T Cell Leukopak Isolation Kit

- 1 Wash the apheresis material with a cell washing instrument to isolate cells and remove platelets. Harvest the cells in a sterile bag and concentrate the cells in separation buffer or complete cell culture media to between 2×10^8 to 4×10^8 cells / mL.
 - a If a cell washing instrument is not to be used, instructions for manual platelet removal can be found in the standard Human T Cell Leukopak Isolation Kit User Guide.
- 2 To enable even mixing, aseptically add air equivalent to 20% volume of the bag.
- 3 Determine volume of antibody cocktail to add to sample: Add 1 mL of Human T Cell Leukopak Biotin Antibody Cocktail for every 10^9 cells.
- 4 Add antibody cocktail aseptically.
- 5 Mix bag by end-over-end rotation at 20 rpm for 15 minutes at room temperature.

Bind Streptavidin Microbubbles

- 6 Determine number of microbubbles to add to sample: Add 12.5 mL of Streptavidin Microbubbles for every 10^9 cells.
Note: Ensure microbubbles are evenly mixed immediately prior to addition.
- 7 Mix samples by end-over-end rotation at 20 rpm for 15 minutes at room temperature.
Note: Use an end-over-end rotator to achieve required mixing.

Separate Cells

- 8 Add additional separation buffer or complete media as desired and separate microbubbles and cells via gravity. Suspend the sample bag from a hanger for 15 minutes to allow all the microbubbles to rise to the top of the sample.
- 9 Leaving the microbubbles behind, aseptically drain the untouched cellular material into a bag or collection vessel, either manually or with a cell washing instrument.
Note: For increased efficiency during manual draining, suspend the bag > 50 cm above transfer bag.
- 10 The untouched cells are the negatively-selected cells of interest, ready for use in downstream assays or expansion.

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.

Control Number UG13210221GB05

Patent No. 11,291,931

The purchase and use of Akadeum Life Sciences products are subject to the terms and conditions at akadeum.com/terms/.

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