Microbubble Leukopak Human T Cell Isolation Kit (Cat.# 13210-221)

BACS™ Microbubble Protocol

The Microbubble Leukopak Human T Cell Isolation Kit was developed with BACS™ Microbubbles to isolate pristine and untouched T cells from leukopak material. Non-T cells are targeted and removed with antibodies recognizing CD14, CD16, CD19, CD20, CD36, CD56, CD123, and CD235ab via negative selection. Isolated T cells are suitable for flow cytometry, molecular assays, activation and expansion, cell culture, or other functional studies.

Name	Format	Quantity	Storage
BACS™ Streptavidin Microbubbles	In buffer with 0.09% sodium azide.	157 mL	2-8 °C
Separation Buffer	Ca^{2+} and Mg^{2+} —free PBS containing 2 mM EDTA and 0.5% biotin-free BSA.	500 mL	2-8 °C
Microbubble Leukopak Human T Cell Biotin Antibody Cocktail	Monoclonal antibodies in PBS with sodium azide.	12.5 mL	2-8 °C
Microbubble Separation Tubes	Sterilized and individually wrapped.	5	Ambient

Additional Supplies:

1. 20 rpm tube rotator for mixing (e.g., Thermo Scientific cat#: 88881002)
2. Centrifuge (swinging bucket rotor strongly preferred)
3. Optional, Vacuum aspirator
4. 50 mL centrifuge tubes

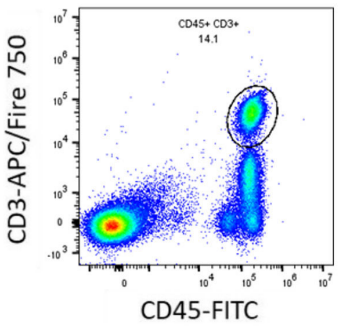
Before You Begin:

- This protocol has been optimized for leukopak material. 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. Cleanse exterior bottom third of Microbubble Separation Tubes with 70% ethanol prior to draining cells (step 19).
- This protocol is designed for starting samples containing 0.5 x 10^9 – 2.5 x 10^9 total cells. For samples outside of this range, please contact techsupport@akadeum.com

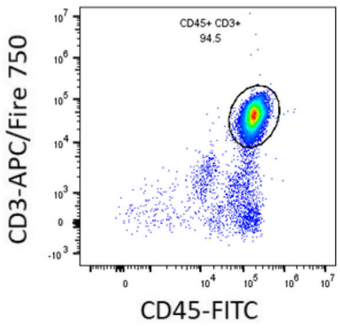
Representative Leukopak Human T Cell Isolation:

Untouched human T cells were isolated from a leukopak. Isolated cells were labeled with CD45-FITC and CD3-APC/Fire750. The fluorescently labeled cells were analyzed by flow cytometry. Debris and dead cells were excluded from analysis.

Pre-Isolation:
14% Human T Cells



Post-Isolation:
95% Human T Cells



Experimental Setup:

Sample Size (Before Beginning)	Initial Sample Volume (Step 9)	Separation Tube (Step 10)	Antibody Cocktail (Step 11)	BACS™ Microbubbles (Step 13)	Final Volume (Step 14)
x10^9 cells	per 10^9 cells		per 10^9 cells	per 10^9 cells	Separation Buffer
0.5 - 2.5	3 mL (333 x 10^6 cells /mL)	50 mL	1 mL	12.5 mL	Fill to 45 mL

Prepare Sample:

1. Aliquot up to 25 mL of leukopak material in 50 mL conical tubes.
2. Bring tubes to 45 mL with Separation Buffer and centrifuge at 400 x g for 10 min (brake optional).
3. Remove the supernatant and resuspend each cell pellet in 1 mL of Separation Buffer.
4. Combine cell suspensions from two tubes into one. Repeat for remaining tubes. If starting with an odd number of tubes, please divide evenly.
5. Bring tubes to 45 mL with Separation Buffer and centrifuge at 400 x g for 10 min (brake optional).
6. Remove supernatant and resuspend each cell pellet in 1 mL of Separation Buffer.
7. Combine all cell suspensions into 1 tube.
   Note: It is recommended to wash remaining tubes with 1mL of separation buffer, and combine with cell suspension to prevent loss of cells.
8. Count total cells (WBC + RBC).
   Note: A 1:100 - 1:1000 dilution is typically needed for accurate counting.
9. Dilute suspension with Separation Buffer to 333 x 10^6 cells / mL (final of 3 mL per 10^9 total cells).
10. Transfer 0.5 x 10^9 – 2.5 x 10^9 cells (1.5 – 7.5 mL of cell suspension) to Microbubble Separation Tube.

Label Cells

11. Add 1 mL of Microbubble Leukopak Human T Cell Biotin Antibody Cocktail per 1 x 10^9 total cells (WBC + RBC) as indicated in the table above. Gently mix samples and incubate for 10 min at room temperature.

Bind BACS™ Microbubbles:

12. Resuspend BACS™ Streptavidin Microbubbles by pipetting or inverting bottle by hand.

    Note: It is critical that BACS™ Streptavidin Microbubbles are thoroughly resuspended immediately prior to addition to each sample. Mix to ensure a homogeneous suspension is created.

13. Add 12.5 mL of BACS™ Streptavidin Microbubbles per 1 x 10^9 total cells (WBC + RBC) as indicated in the table above.
14. Add Separation Buffer to achieve a final volume of 45 mL. Close separation tube by moving cap to locked position.
15. Mix samples on a rotator at 20 rpm for 10 min at room temperature.

Separate Cells:

16. Centrifuge samples at 100 x g for 2 min (with brake).
    Note: A swinging bucket rotor centrifuge is recommended.
17. Using the knob on the cap of the Separation Tube, complete 2-3 back and forth rotations to loosen the cell pellet.
18. Open a clean 50 mL conical tube and place it in a tube rack.
19. First, cleanse exterior bottom third of the Microbubble Separation Tube with 70% ethanol, then hold over the open 50 mL conical and turn the knob on the cap to the drain position to initiate draining of the cell fraction.
20. Centrifuge sample tube at 400 x g for 5 min (with brake).
21. Vacuum aspirate the supernatant, taking care not to disturb the cell pellet containing the purified T cells.
22. Resuspend cell pellet in desired buffer or media for downstream use.

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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 here for additional product information about our Microbubble Leukopak Human T Cell Isolation Kit

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