Human CD4+ T Cell Isolation Kit
with Akadeum BACS™ Microbubbles

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PRODUCT OVERVIEW

- Akadeum's Human CD4+ T Cell Isolation Kit uses streptavidin-conjugated BACS™ microbubbles to negatively select CD4+ T cells from peripheral blood mononuclear cells (PBMCs).
- Non-CD4+ T cells (unwanted cells) are labelled with biotinylated antibodies targeting CD8, CD11b, CD14, CD16, CD19, CD36, CD56, CD123, CD235a, and TCR-γδ, and subsequently mixed with microbubbles.
- Once bound to unwanted cells, microbubbles enable rapid cell removal without a column or magnet.
- High purity human CD4+ T cells are left untouched and ready for downstream applications.

Components of Human CD4+ T Cell Isolation Kit (#10023):

In The Box

BACS™ Streptavidin Microbubbles

Human CD4+ T Cell Antibody Cocktail

Packaged Separately

Separation Buffer

Ca^{2+} and Mg^{2+} free PBS containing 2 mM EDTA, 0.5% biotin free BSA, and 0.09% sodium azide

5 mL Eppendorf Tubes

Additional recommended supplies:

Low retention 1 mL pipet tips

VWR Part #89174-530

Centrifuge with swinging bucket rotor

Storage: Store microbubbles and antibody cocktail at 4°C

Cell Type: Naive/resting CD4+ T cells

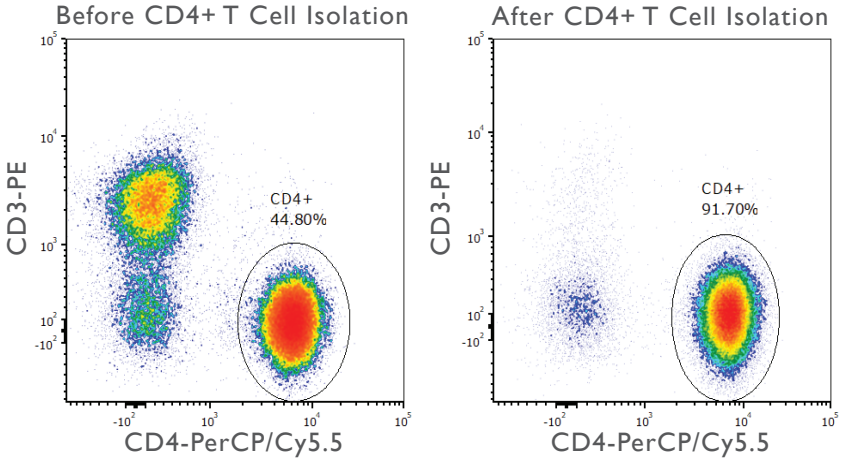
Sample Species and Source: Human PBMCs

Cell Separation Method: Negative selection

Capacity: 1 billion cells

EXAMPLE OF A HUMAN CD4+ T CELL ISOLATION

Isolation of human CD4+ T cells from PBMCs was performed using Akadeum Life Sciences Human CD4+ T Cell Isolation Kit (#10023). Cells were labeled with CD4-PerCP/Cy5.5 and CD3-PE. Dead cells and debris were excluded from analysis.



GENERAL NOTES

For tips concerning sample preparation, cell labeling and separation, visit **www.akadeum.com/technology/**

For product-specific background information and applications of this product, refer to the respective product page at **www.akadeum.com/products/**

SAFETY INFORMATION

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Before use, please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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Protocol: Human CD4+ T Cell Isolation

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The protocol below is for samples containing between 10^7 and 10^8 cells. If a sample is larger than 10^8 cells, split the sample into two. For a protocol optimized for samples fewer than 10^7 cells, contact us: **info@akadeum.com**

Note: Work quickly using pre-chilled Separation Buffer (2-8°C)

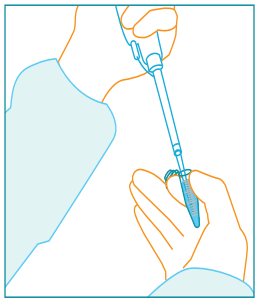
Prepare your cells

1. Begin with human PBMCs.
2. Centrifuge PBMC suspension (5 min, 400 x g), aspirate supernatant, and wash once with 5 mL of Separation Buffer.
3. Centrifuge samples (5 min, 400 x g) and aspirate supernatant.
4. Resuspend cells at 1 x 10^7 cells per 50 µL of Separation Buffer (see *Table 1*) and transfer to 5 mL Eppendorf tubes.

Label cells

5. Add 10 µL of biotin-antibody cocktail per 1 x 10^7 cells (see *Table 1*). Mix briefly.
6. Incubate sample for 20 min at 4°C.
7. Add 1 mL Separation Buffer and centrifuge (5 min, 400 x g). Aspirate supernatant.
8. Resuspend cells at 1 x 10^7 cells per 50 µL of Separation Buffer (see *Table 1*).

Bind microbubbles

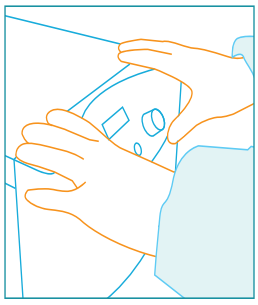


9. Prepare microbubbles by resuspending in solution (microbubble mixture should be a homogenous white solution, *i.e.* look like milk). Vigorously mix or pipet. Immediately proceed to next step.
10. Add 175 µL of microbubbles per 1 x 10^7 cells to first sample (see *Table 1*).
11. Set the pipet volume to ~50% of the total sample volume (cell suspension + microbubbles) and mix with gentle trituration for 30 pipet strokes using a low retention 1000 µL pipet tip (see *Table 1*).

    Note: This pipet setting ensures adequate mixing of microbubbles and cells for binding.
12. Immediately add 3 mL Separation Buffer to sample.

    Note: This faciliatates separation of microbubbles from the cell pellet.
13. Repeat steps 10-12 for remaining samples making sure to **resuspend microbubbles before each sample.**

Separate cells



14. Centrifuge samples (5 min, 400 x g).

    Note: Akadeum recommends using a swinging bucket rotor for this step to facilitate microbubble aspiration.
15. Aspirate off white microbubble layer and supernatant. Take care not to aspirate cell pellet.
16. Resuspend cells at 1 x 10^7 cells per 50 µL of Separation Buffer (see *Table 1*) by pipetting. Transfer cells to a new tube if desired due to residual microbubbles stuck to tube.
17. Continue to flow cytometry or other downstream processing or handling.

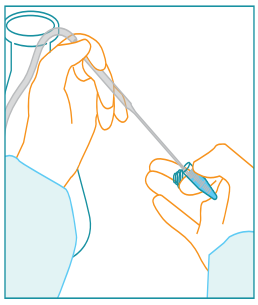


Table 1. Example Volumes For Cell Isolation

Total Cells	Resuspension Volume	Antibody	Microbubbles	Mixing Volume
1 x 10^7	50µL	10µL	175µL	113µL
5 x 10^7	250µL	50µL	875µL	563µL
1 x 10^8	500µL	100µL	1750µL	1000µL

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