Mouse CD4+ T Cell Isolation Kit

with Akadeum BACS™ Microbubbles

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Components of Mouse CD4+ T Cell Isolation Kit (Full Kit #12210-130) (Trial Kit #22210-320):

IN THE BOX:

1. BACS™ Streptavidin Microbubbles
2. Mouse CD4+ T Cell Antibody Cocktail

PACKAGED SEPERATELY:

1. Separation Buffer Ca^{2+} and Mg^{2+} free PBS containing 2 mM EDTA, 0.5% biotin-free BSA, and 0.09% sodium azide
2. 5 mL Eppendorf Tubes

Additional Recommended Supplies:

1. Low retention 1 mL pipet tips VWR Part #89174-530
2. Centrifuge with swinging bucket rotor
3. Vacuum aspirator

PRODUCT OVERVIEW

- Akadeum's Mouse CD4+ T Cell Isolation Kit uses streptavidin- conjugated BACS™ microbubbles biotinylated antibodies to negatively select CD4+ T cells from mouse splenocytes.
- Non-CD4+ T cells (unwanted cells) are labelled with biotinylated antibodies targeting B220 (CD45R), CD8a, CD11b, CD11c, CD24, CD49b, Gr-1, TCR-γδ and TER-119, and subsequently mixed with microbubbles.
- Once bound to unwanted cells, microbubbles enable rapid cell removal without a column or magnet.
- High purity mouse CD4+ T cells are left untouched and ready for downstream applications.

Storage: Store microbubbles, antibody cocktail, and Separation Buffer at 4°C

Cell Type: Naive/resting CD4+ T cells

Sample Species and Source: Mouse spleen. Lysis of red blood cells prior to use is strongly encouraged.

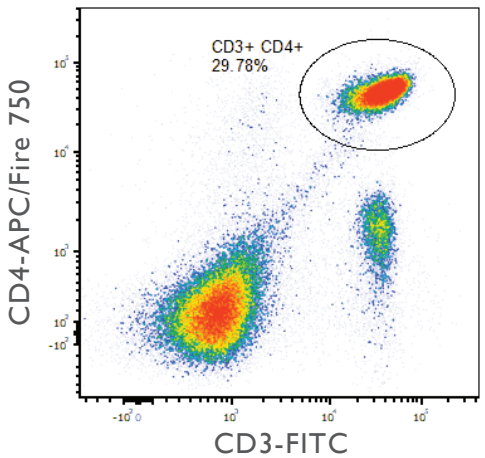
Cell Separation Method: Negative Selection

Capacity: Full Kit = 1 billion cells; Trial Kit = 300 million cells

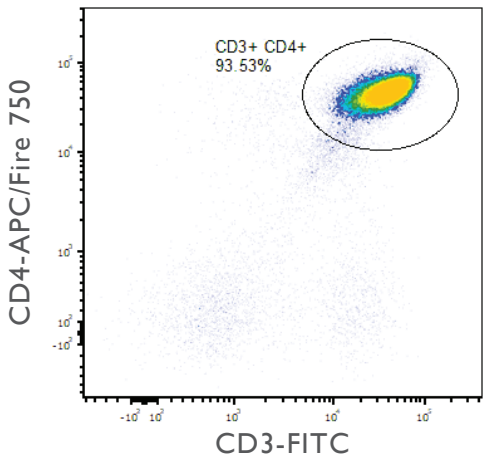
EXAMPLE OF A MOUSE CD4+ T CELL ISOLATION

Fresh murine CD4+ T cells were isolated from a heterogenous splenocyte population using Akadeum Life Sciences Mouse CD4+ T Cell Isolation Kit (#12210-130). CD4+ T cells were labeled with CD3-FITC and CD4-APC/Fire 750. Dead cells and debris were excluded from analysis.

Before CD4+ T Cell Isolation



After CD4+ T Cell Isolation



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.

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/

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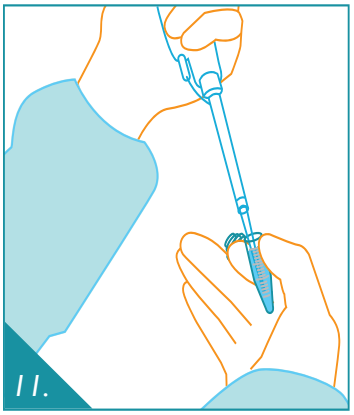
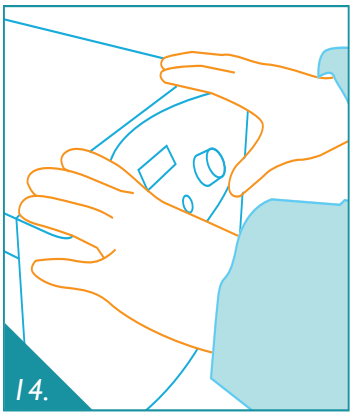
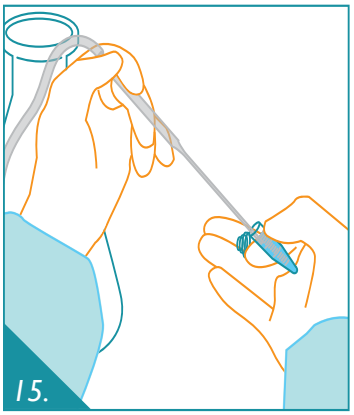
Protocol: Mouse CD4+ T Cell Isolation

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. Homogenize mouse spleen and lyse red blood cells.
2. Centrifuge splenocyte 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 Table1) and transfer to 5 mL Eppendorf tubes.

LABEL CELLS

5. Add 15 µL of biotin-antibody cocktail per 1 x 10^7 cells (see Table1). 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 Table1).

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 150 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 Table1).

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

    Note: This facilitates 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. Use vacuum aspirator to 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 Table1) 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	15µL	150µL	100µL
5 x 10^7	250µL	75µL	750µL	500µL
1 x 10^8	500µL	150µL	1500µL	1000µL

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