Mouse Naïve CD4+T Cell Isolation Kit

with Akadeum BACS™ Microbubbles

AKADEUM LIFE SCIENCES

Components of Mouse Naïve CD4+ T Cell Isolation Kit (#12210-150; Trial Kit #22210-150):

IN THE BOX:

1. Mouse naïve CD4+ T cell biotin-antibody cocktail
2. Streptavidin BACS™ microbubbles

PACKAGED SEPERATELY:

1. Separation Buffer Ca^{2+}/Mg^{2+} free PBS containing 2 mM EDTA, 0.5% biotin free BSA, 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 Naïve CD4+ T Cell Isolation Kit uses streptavidin-conjugated BACS™ microbubbles to negatively select naïve CD4+ T cells from mouse splenocytes.
- Non-naïve CD4+ T cells (unwanted cells) are labelled with biotinylated antibodies targeting B220 (CD45R), CD8a, CD11b, CD11c, CD24, CD25, CD44, 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 naïve CD4+ T cells are left untouched and ready for downstream applications.

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

Cell Type: Naïve T cells

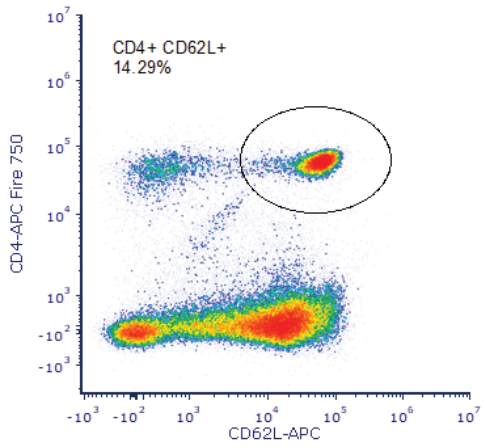
Sample Species and Source: Mouse spleen. Lysis of red blood cells prior to use is strongly encouraged.

Cell Separation Method: Negative selection

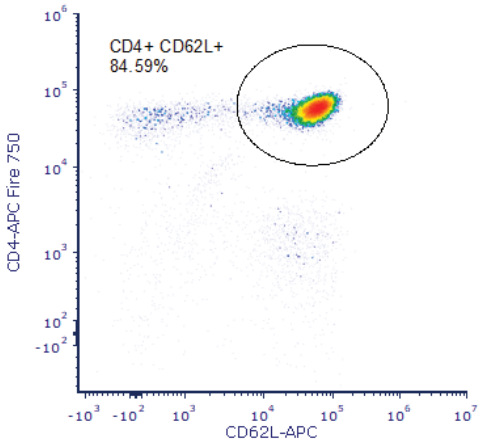
Capacity: Full Kit = 1 billion cells; Trial Kit = 300 million cells

EXAMPLE OF SEPARATION USING AKADEUM MOUSE NAÏVE CD4+ T CELL ISOLATION KIT

Fresh murine naïve CD4+ T cells were isolated from a heterogenous splenocyte population. Dead cells and debris were excluded from analysis and naïve CD4+ T cells were labeled with CD62L-APC and CD4-APC Fire 750.



Control



Post-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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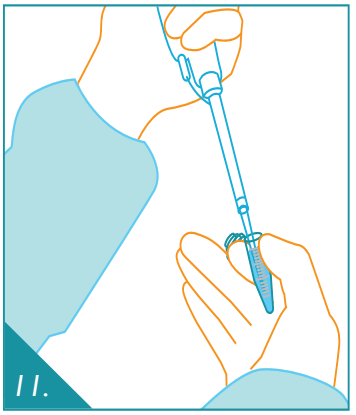
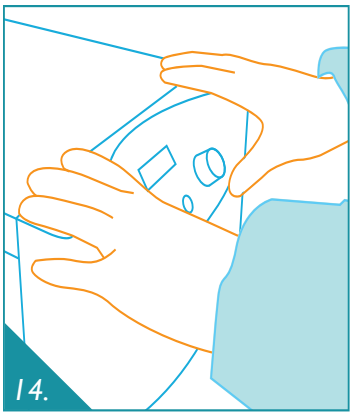
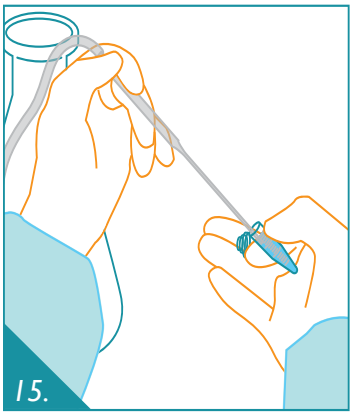
Protocol: Mouse Naïve CD4+ T Cell Isolation

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: ***techsupport@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 mouse 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 cell pellet in separation buffer at 1×10^7 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-labeled antibody cocktail per 1×10^7 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 1×10^7 cells per 50 µL of separation buffer (see Table 1).

BIND MICROBUBBLES

9. Prepare microbubbles by resuspending microbubbles in solution (mixture should be a homogeneous white solution, i.e. look like milk). Vigorously mix or pipet. Immediately proceed to Step 10.
10. Add 131 µL of microbubbles per 1×10^7 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: *Mixing at 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 the 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" with "Use vacuum aspirator to aspirate off white microbubble layer and supernatant. Take care not to aspirate cell pellet.
16. Resuspend cell pellet at 1×10^7 cells per 50 µL of separation buffer by pipetting. Transfer cells to a clean 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 Mouse Naïve CD4+ T Cell Isolation

Total Cells	Resuspension Volume	Antibody	Microbubbles	Mixing Volume
1×10^7	50µL	10µL	131µL	91µL
5×10^7	250µL	50µL	656µL	453µL
1×10^8	500µL	100µL	1313µL	907µL

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