

# Mouse Naïve T Cell Isolation Kit

with Akadeum BACS™ Microbubbles



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## Components of Mouse Naïve T Cell Isolation Kit (#12210-160; Trial Kit #22210-160):

### IN THE BOX:

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

### PACKAGED SEPERATELY:

1. Separation Buffer  $\text{Ca}^{2+}/\text{Mg}^{2+}$  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 T Cell Isolation Kit uses streptavidin-conjugated BACS™ microbubbles to negatively select naïve T cells from mouse splenocytes.
- > Non-naïve T cells (unwanted cells) are labelled with biotinylated antibodies targeting B220 (CD45R), CD11b, CD11c, CD24, CD25, CD44, CD49b, Gr-1 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 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

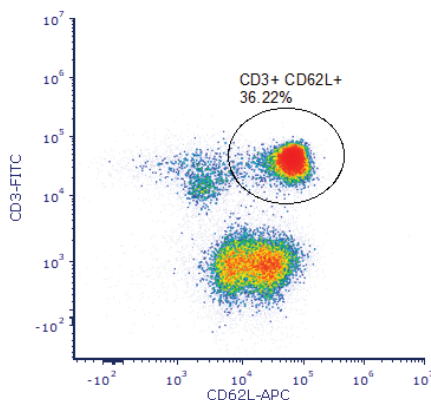
**Sample Species and Source:** Mouse spleen. Lysis of red blood cells prior to use is strongly encouraged.

**Cell Separation Method:** Negative selection

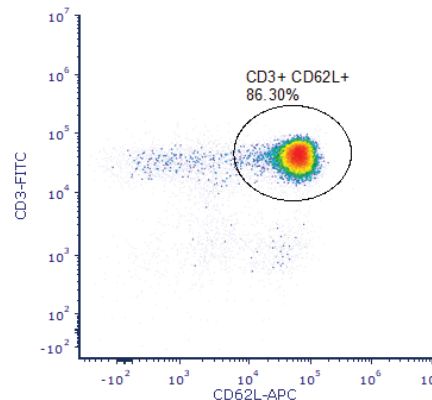
**Capacity:** Full Kit = 1 billion cells; Trial Kit = 300 million cells

## EXAMPLE OF A MOUSE NAÏVE T CELL ISOLATION

Fresh murine naïve T cells were isolated from a heterogenous splenocyte population. Dead cells and debris were excluded from analysis and naïve T cells were labeled with CD62L-APC and CD3-FITC



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/](http://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/](http://www.akadeum.com/products/)

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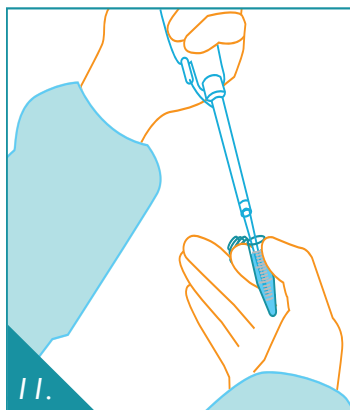


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## Protocol: Mouse Naïve T Cell Isolation

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](mailto: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 × g), aspirate supernatant, and wash once with 5 mL of separation buffer.
3. Centrifuge samples (5 min, 400 × g) and aspirate supernatant.
4. Resuspend cell pellet in separation buffer at  $1 \times 10^7$  cells per 50  $\mu$ L of separation buffer (see Table 1) and transfer to 5 mL Eppendorf tubes.

### LABEL CELLS

5. Add 10  $\mu$ L of biotin-labeled antibody cocktail per  $1 \times 10^7$  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 × g). Aspirate supernatant.
8. Resuspend cells at  $1 \times 10^7$  cells per 50  $\mu$ L 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 123  $\mu$ L of microbubbles per  $1 \times 10^7$  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  $\mu$ L 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.

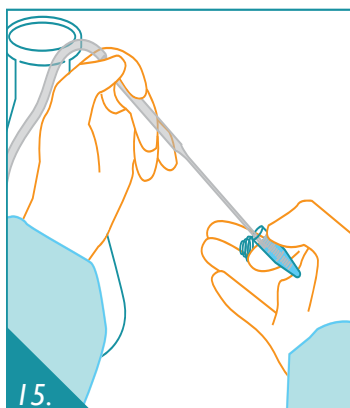
13. Repeat steps 10-12 for remaining samples **making sure to resuspend microbubbles before each sample.**

### SEPARATE CELLS

14. Centrifuge samples (5 min, 400 × 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 cell pellet at  $1 \times 10^7$  cells per 50  $\mu$ L 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



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Table 1. Example Volumes For Mouse Naïve T Cell Isolation

Total Cells	Resuspension Volume	Antibody	Microbubbles	Mixing Volume
$1 \times 10^7$	50 $\mu$ L	10 $\mu$ L	123 $\mu$ L	87 $\mu$ L
$5 \times 10^7$	250 $\mu$ L	50 $\mu$ L	613 $\mu$ L	432 $\mu$ L
$1 \times 10^8$	500 $\mu$ L	100 $\mu$ L	1225 $\mu$ L	863 $\mu$ L