

Mouse B Cell Isolation Kit

with Akadeum BACS™ Microbubbles



PRODUCT OVERVIEW

- > Akadeum's Mouse B Cell Isolation Kit uses streptavidin-conjugated BACS™ microbubbles to negatively select B cells from mouse splenocytes.
- > Non-B cells (unwanted cells) are labelled with biotinylated antibodies targeting CD3, CD4, CD8a, CD11b, CD11c, 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 B cells are left untouched and ready for downstream applications.

Components of Mouse B Cell Isolation Kit (#10019):

In The Box

BACS™ Streptavidin Microbubbles
Mouse B Cell Antibody Cocktail

Packaged Separately

Separation Buffer
Ca²⁺ and Mg²⁺ 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 B cells

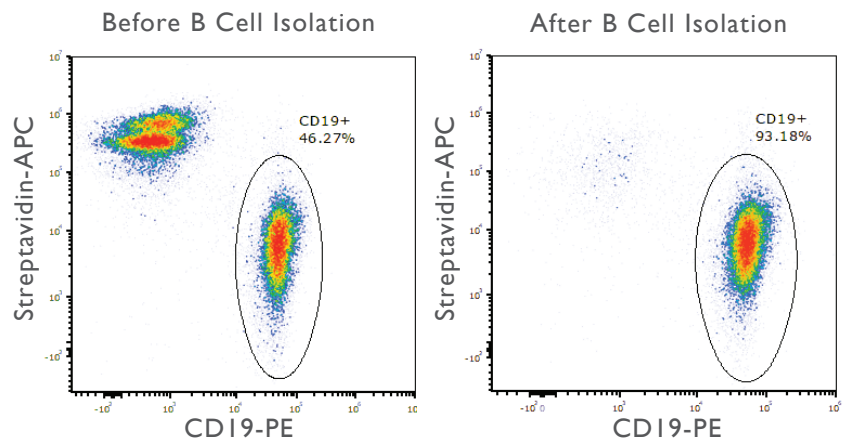
Sample Species and Source: Mouse spleen. Lysis of red blood cells prior to use is strongly encouraged.

Cell Separation Method: Negative selection

Capacity: 1 billion cells

EXAMPLE OF A MOUSE B CELL ISOLATION

Fresh murine B cells were isolated from a heterogenous splenocyte population using Akadeum Life Sciences Mouse B Cell Isolation Kit (#10019). B cells were labeled with CD19-PE and microbubble targeted cells were labeled with Streptavidin-APC. 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.

Protocol: Mouse B 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)

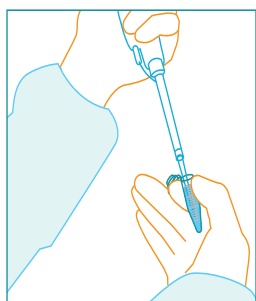
Prepare your cells

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 1×10^7 cells per 50 μ L of Separation Buffer (see Table I) and transfer to 5 mL Eppendorf tubes.

Label cells

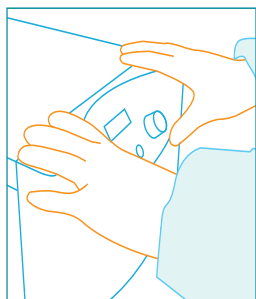
5. Add 10 μ L of biotin-antibody cocktail per 1×10^7 cells (see Table I). Mix briefly.
6. Incubate sample for 20 min at 4°C.
7. Add 1 mL Separation Buffer and centrifuge (5 min, 400 x g). Aspirate supernatant.
8. Resuspend cells at 1×10^7 cells per 50 μ L of Separation Buffer (see Table I).

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 80 μ L of microbubbles per 1×10^7 cells to first sample (see Table I).
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 μ L pipet tip (see Table I).
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. Aspirate off white microbubble layer and supernatant. Take care not to aspirate cell pellet.
16. Resuspend cells at 1×10^7 cells per 50 μ L of Separation Buffer (see Table I) 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.

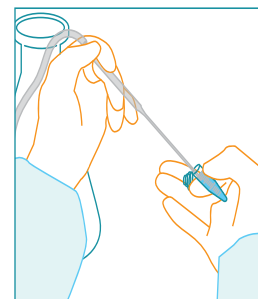


Table I. Example Volumes For Cell Isolation

Total Cells	Resuspension Volume	Antibody	Microbubbles	Mixing Volume
1×10^7	50 μ L	10 μ L	80 μ L	65 μ L
5×10^7	250 μ L	50 μ L	400 μ L	325 μ L
1×10^8	500 μ L	100 μ L	800 μ L	650 μ L