Mouse B Cell Isolation Kit (Cat.12210-110)

BACS™ Microbubbles Protocol

Isolate untouched B cells from mouse splenocytes via Buoyancy Activated Cell Sorting (BACS). This kit can be used to target and remove non-B cells with antibodies recognizing CD3, CD4, CD8a, CD11b, CD11c, CD49b, CD90.2, CD105, Gr1, and Ter119. Isolated B cells are suitable for flow cytometry, molecular assays, cell culture, and other functional studies. Processing capacity 1 x 10^9 cells.

Name	Format	Quantity	Storage
BACS™ Streptavidin Microbubbles	In buffer with 0.09% sodium azide.	10.5 mL	2-8 °C
Mouse B Cell Biotin Antibody Cocktail	Monoclonal antibodies in PBS with sodium azide.	1050 µL	2-8 °C
Separation Buffer	Ca^{2+} and Mg^{2+} –free PBS containing 2 mM EDTA and 0.5% biotin-free BSA.	200 mL	2-8 °C
5 mL Tubes	Bag of tubes	20 tubes	RT

Additional Supplies:

1. 20 rpm tube rotator for mixing (e.g., Thermo Scientific cat#: 88881002)
2. Centrifuge (swinging bucket rotor strongly preferred)
3. Vacuum aspirator
4. 30 µm cell strainer (optional)

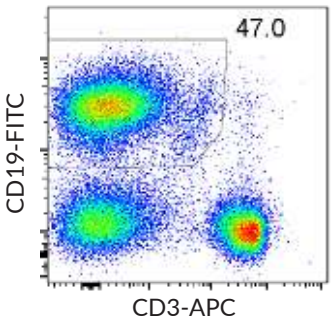
Before You Begin:

- This protocol has been optimized for splenocytes as the starting material. If working with other sample types, please contact techsupport@akadeum.com.
- For optimal results, homogenize mouse spleens and lyse red blood cells in the sample prior to separation.
- Separation Buffer is azide-free. Cell isolation should be conducted under aseptic conditions.
- For optimal results, prior to cell separation, filter samples through a 30 µm cell strainer to obtain a single-cell suspension.
- For tips on how to vacuum aspirate the BACS™ Microbubble layer, see video: https://www.akadeum.com/videos/aspiration
- This protocol is designed for starting samples containing 1 x 10^7 – 24 x 10^7 total cells. Samples with > 24 x 10^7 should be divided across multiple tubes. For samples <1x10^7, please contact techsupport@akadeum.com

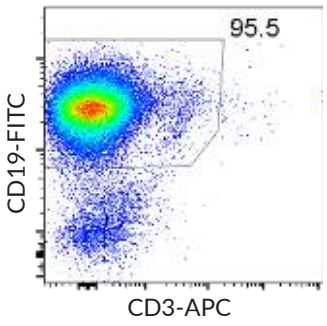
Representative B Cell Isolation:

Untouched B cells were isolated from splenocytes. Isolated cells were labeled with CD19-FITC and CD3-APC. The fluorescently labeled cells were analyzed by flow cytometry. Debris and dead cells were excluded from analysis.

Pre-Isolation:
47% B Cells



Post-Isolation:
96% B Cells



Experimental Setup:

Sample Size	Tube Size	Sample Volume (Step 2)	Antibody Cocktail (Step 4)	BACS™ Microbubbles (Step 6)	Final Volume (Step 7)
($1x10^7$ cells)		per ($1x10^7$ cells)	per ($1x10^7$ cells)	per ($1x10^7$ cells)	Separation Buffer
1 - 7	1.5 mL	30 µL	10 µL	100 µL	Fill to 1.2 mL
> 7 - 24	5.0 mL	30 µL	10 µL	100 µL	Fill to 4.0 mL

Prepare Cells:

1. Count and wash cells.
2. Resuspend cell pellet in 30 µL of Separation Buffer per 1 x 10^7 cells, as indicated in the table above.
3. Transfer cell suspension to a 1.5 or 5 mL tube, as indicated in the table above. Divide or aliquot sample to be within the cell number ranges indicated in the table above.

Label Cells:

4. Add 10 µL of Mouse B Cell Isolation Biotin Antibody Cocktail per 1 x 10^7 total cells as indicated in the table above. Gently mix samples and incubate for 10 min at room temperature (or at 4˚C).

Bind BACS™ Microbubbles:

5. Resuspend BACS™ Microbubbles by pipetting or inverting by hand.

Note: It is critical that BACS™ Microbubbles are thoroughly resuspended immediately prior to addition to each sample. Resuspension can be achieved by pipetting with a 1 mL pipette 2-3 times, followed by inverting multiple times to create a homogeneous suspension.

6. Add 100 µL of BACS™ Microbubbles per 1 x 10^7 total cells to the labeled sample as indicated in the table above.
7. Add Separation Buffer to achieve a final volume of 1.2 or 4.0 mL, as indicated in the table above.
8. Mix samples on a rotator at 20 rpm for 10 min at room temperature (or at 4˚C).

Separate Cells:

9. Centrifuge samples at 400 x g for 5 min.

Note: A swinging bucket rotor centrifuge is recommended.

10. Vacuum aspirate the BACS™ Microbubble layer and supernatant, taking care not to disturb the cell pellet. Once BACS™ Microbubbles have been aspirated, the supernatant may be removed by pipette.

Note: For tips on how to remove BACS™ microbubbles, see video: https://www.akadeum.com/videos/aspiration

11. Resuspend cell pellet in desired buffer or media and transfer to clean tube.

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Safety Information

For research use only. Not intended for any animal or human therapeutic or diagnostic use. For information regarding hazards and safe handling practices, please consult the Safety Data Sheet.

Visit us here for how-to videos, additional product information, and tech support

Version #: P-0005-0001
Patent No. 11,291,931