Dead Cell Removal Microbubble Kit (Cat.11510-211)

(Cayman Item No. 44554)

BACS™ Microbubbles Protocol

Akadeum's targeted removal of dead cells is achieved through the selective capture of cells with exposed phosphatidylserine (PS) using Annexin V conjugated BACS™ microbubbles. Once mixed with the sample, the BACS™ Dead Cell Removal microbubbles capture dead cells and float them to the surface for removal, leaving the untouched cells of interest happy, healthy, and ready for downstream use.

Name	Format	Quantity	Storage
BACS™ Dead Cell Removal Microbubbles (Blue Cap)	In dead cell removal binding buffer.	1 x 1 mL	2-8 °C
Dead Cell Removal Binding Buffer	Binding buffer containing 0.2% biotin-free BSA.	2 x 50 mL	2-8 °C
Calcium Chloride (Orange Cap)	1 M $CaCl_2$	1 x 500 µL	-20 °C

Additional Supplies:

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

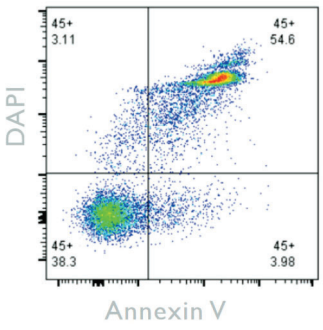
Before You Begin:

- This protocol has been optimized for samples with 35% or greater starting viability. For samples below 35% viability, please contact techsupport@akadeum.com
- For optimal results, prior to cell separation, filter samples through a 30 µm cell strainer to obtain a single-cell suspension.
- Dead Cell Removal Binding Buffer is azide-free. Cell isolation should be conducted under aseptic conditions.
- 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 0.5 x 10^6 – 25 x 10^6 total cells (live and dead). For samples outside of this range, please contact techsupport@akadeum.com

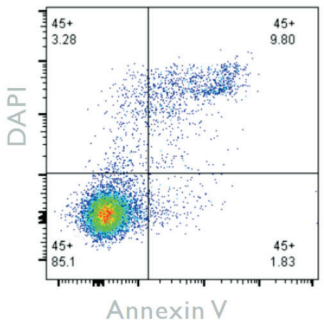
Representative Dead Cell Removal :

Example of dead cell removal from apopotic mouse splenocytes. Enriched viable cells were stained with DAPI and Annexin V. The fluorescently labeled cells were analyzed by flow cytometry.

Before Dead Cell Removal:
38% Viable



After Dead Cell Removal:
85% Viable



Experimental Setup:

Sample Size (x10^6 cells)	DCR Binding Buffer Resuspension (Step 2)	1M $CaCl_2$ (Step 4)	BACS™ Microbubbles (Step 6)	Additional DCR Binding Buffer (Step 7)
0.5	10 µL	2 µL	10 µL	980 µL
1	20 µL	2 µL	20 µL	960 µL
5	100 µL	2 µL	100 µL	800 µL
10	200 µL	2 µL	200 µL	600 µL
25	500 µL	2 µL	500 µL	0 µL

Prepare Cells:

1. Count total cells (live and dead) and centrifuge cells.
2. Resuspend cell pellet in 20 µL of DCR Binding Buffer per 1 x 10^6 total cells, as indicated in the table above.
3. If not in 1.5 mL tube, transfer to 1.5 mL tube. Divide or aliquot sample to be within the cell number ranges indicated in the table above.

Bind BACS™ Microbubbles:

4. Add 2 µL of 1 M $CaCl_2$ (Orange Cap). Refer to table 1.

   Note: For best results, after addition of $CaCl_2$ it is important to continue through the protocol without interruption.

5. Resuspend BACS™ Microbubbles (Blue Cap) by pipetting or inverting by hand. Proceed immediately to step 6.

   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 or inverting multiple times.

6. Add 20 uL of BACS™ Microbubbles (Blue Cap) per 1 x 10^6 total cells to the labeled sample as indicated in the table above.
7. Add DCR Binding Buffer to achieve a final volume of 1000 µL, as indicated in the table above.
8. Mix samples on a rotator at 20 rpm for 15 min at ambient 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

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