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LIFE SCIENCES

Human PBMC Leukopak Isolation Kit (Cat.# 13210-223)

BACS™ Microbubble Protocol

This protocol is designed for starting samples containing 0.5 – 4.2 x 10^9 total cells per 50 mL tube. The Microbubble Leukopak Human Peripheral Blood Mononuclear Cell (PBMC) Isolation Kit was developed with BACS™ Microbubbles to isolate PBMCs from human leukopak material via negative selection. Non-PBMCs are targeted and removed with antibodies recognizing CD66b, CD123, and CD235ab, leaving PBMCs untouched. Isolated PBMCs are suitable for flow cytometry, molecular assays, activation and expansion, cell culture, and other functional studies.

Name	Format	Quantity	Storage
BACS™ Streptavidin Microbubbles	In buffer	62.5 mL	2-8 °C
Separation Buffer	Ca^{2+} and Mg^{2+} free PBS containing 2 mM EDTA and 0.5% biotin-free BSA.	500 mL	2-8 °C
Microbubble Leukopak Human PBMC Biotin Antibody Cocktail	Monoclonal antibodies in Separation Buffer with <0.09% sodium azide	12.5 mL	2-8 °C
Microbubble Separation Tubes	Sterilized and individually wrapped.	3 Tubes	Ambient

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 (optional but strongly recommended)
4. 50 mL centrifuge tubes
5. Additional Microbubble Separation Tubes (If working with sample sizes <4.2 x 10^9 cells, Cat#: 30004-050-1-10)

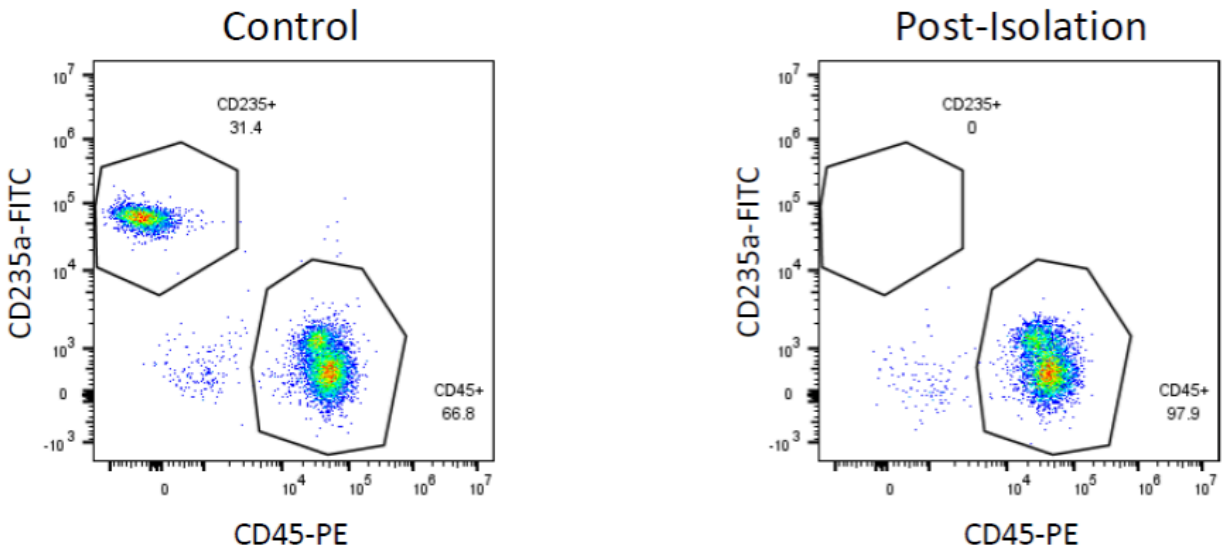
Before You Begin:

- This protocol has been optimized for leukopak material. For alternative starting materials, please contact techsupport@akadeum.com.
- For maintenance of sterility, cell isolation should be conducted in a biosafety cabinet using aseptic technique. Cleanse exterior bottom third of Microbubble Separation Tubes with 70% ethanol or isopropanol prior to draining cells (step 19). This kit is designed for 1.25 x 10^{10} total cells, white blood cells (WBC) and red blood cells (RBC) combined.
- This protocol is designed for starting samples containing 0.5 – 4.2 x 10^9 total cells per Separation Tube. For samples outside of this range, please contact techsupport@akadeum.com.
- It is essential to review the accompanying insert on how to use Microbubble Separation Tubes.

Human Leukopak PBMC Isolation:

Leukopak samples contain a milieu of immune cells (i.e., white blood cells), plasma, platelets, and red blood cells. The Microbubble Leukopak Human PBMC Isolation Kit uses a negative selection to enrich leukopak PBMCs by removing unwanted cell populations.

These unwanted cells are labeled with an optimized cocktail of biotinylated antibodies targeting CD66b, CD123, and CD235ab. BACS™ Streptavidin Microbubbles are mixed into the sample where they bind to the labeled cells and gently float them to the surface. Untouched PBMCs in their original state are subsequently harvested. Isolated PBMCs are suitable for flow cytometry, molecular assays, activation and expansion, cell culture, or other functional studies.



Experimental Setup:

Sample Size* (Before Beginning)	Initial Sample Volume (Step 9)	Antibody Cocktail (Step 11)	BACS™ Microbubbles (Step 13)	Final Volume (Step 14)
x10^9 cells	per 10^9 cells	per 10^9 cells	per 10^9 cells	Separation Buffer
0.5 - 4.2	3 mL (3.33 x 10^8 cells /mL)	1 mL	5 mL	Fill to 45 mL

Note: This is the sample size ranger per tube. If working with >4.2 x 10^9 cells, split sample into multiple Microbubble Separation Tubes.

Prepare Sample (Remove Platelets):

Note: A platelet removal protocol on a cell washing instrument can be used in place of steps 1 through 7.

1. Aliquot up to 25 mL of leukopak material in 50 mL conical tubes (not Microbubble Separation Tubes).
2. Bring tubes to 45 mL with Separation Buffer and centrifuge at 400 x g for 10 min (with brake).
3. Remove the supernatant and resuspend each cell pellet in 1 mL of Separation Buffer.
4. Combine cell suspensions from two tubes into one. Repeat for remaining tubes. If starting with an odd number of tubes, please divide evenly.
5. Bring tubes to 45 mL with Separation Buffer and centrifuge at 400 x g for 10 min (with brake).
6. Remove supernatant and resuspend each cell pellet in 1 mL of Separation Buffer.
7. Combine all cell suspensions into one tube.
   Note: It is recommended to wash remaining tubes with 1mL of separation buffer and combine with cell suspension to prevent loss of cells.
8. Count total cells (WBC and RBC together)
   Note: A 1:100 – 1:1000 dilution is typically needed for accurate counting. If using an automated cell counter, ensure instrument-specific settings are applied that will incorporate all WBC and RBC, but not any residual platelets, into the count.
9. Dilute suspension with Separation Buffer to 3.33 x 10^8 cells / mL (final of 3 mL per 10^9 total cells).
10. Transfer 0.5 x 10^9 – 4.2 x 10^9 cells (1.5 – 12.6 mL of cell suspension) to Microbubble Separation Tube.
    Note: It is essential to review the product insert regarding how to use the Microbubble Separation Tube.

Label Cells

11. Add 1 mL of Microbubble Leukopak Human PBMC Biotin Antibody Cocktail per 1 x 10^9 total cells (WBC + RBC) as indicated in the table above. Gently mix samples and incubate for 10 min at room temperature.

Bind BACS™ Microbubbles:

12. Resuspend BACS™ Streptavidin Microbubbles by pipetting or inverting bottle by hand.
Note: It is critical that BACS™ Streptavidin Microbubbles are thoroughly resuspended immediately prior to addition to each sample. Mix to ensure microbubbles are taken from a homogeneous white suspension.

13. Add 5 mL of BACS™ Streptavidin Microbubbles per 1 x 10^9 total cells (WBC + RBC) as indicated in the table.

14. Add Separation Buffer to achieve a final volume of 45 mL. Close separation tube by moving cap to locked position.

15. Mix samples on a rotator at 20 rpm for 10 min at room temperature.

Separate Cells:

16. Centrifuge samples at 100 x g for 2 min at room temperature or 4°C (with brake).
Note: A swinging bucket rotor centrifuge is recommended.

17. Using the knob on the cap of the Separation Tube, complete 2-3 back and forth rotations to loosen the cell pellet.

18. Open a clean 50 mL conical tube and place it in a tube rack.

19. First, cleanse exterior bottom third of the Microbubble Separation Tube with 70% ethanol or isopropanol, then hold over the open 50 mL conical and turn the knob on the cap to the drain position to initiate draining of the cell fraction (approximately 35-40 mL). ***Note: It is essential to review the product insert regarding how to use the Microbubble Separation Tube.***

20. Mix dispensed sample using a serological pipette to ensure single-cell suspension.

21. Centrifuge sample tube at 400 x g for 5 min at room temperature or 4°C (with brake).

22. Vacuum aspirate the supernatant, taking care not to disturb the cell pellet containing the purified PBMCs. ***Note: If residual microbubbles were accidentally released into the 50-mL conical during step 19, they can be easily removed at this stage along with the supernatant.***

23. Resuspend cell pellet in desired buffer or media for downstream use.

Example Sample Compositions

Sample Size (x 10^9 total cells)	Sample Volume (mL)	Antibody Cocktail Volume (mL)	BACS™ Microbubbles	Additional Separation Buffer (mL)	Final Microbubble Mixing Volume (mL)
0.5 – 4.2 x 10^9 cells	**3 mL per 10^9 cells**	**1 mL per 10^9 cells**	**15 mL per 10^9 cells**	**Fill to 45 mL**	**45 mL**
0.5	1.5	0.5	2.5	40.5	45
1.5	4.5	1.5	7.5	31.5	45
3.0	9.0	3.0	15.0	18	45
4.2	12.6	4.2	21.0	7.2	45

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. BSA in the Separation Buffer is made and sourced in the USA, free of TSE and BSE.

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