Human PBMC Leukopak Isolation Kit, Research Scale (Cat.# 13210-223T)

BACS™ Microbubble Protocol

The Human PBMC (Peripheral Blood Mononuclear Cell) Leukopak Isolation Kit was developed with BACS™ microbubbles to isolate untouched PBMCs from human leukopak material. Non-PBMCs are targeted and removed with antibodies recognizing CD66b, CD123, and CD235ab, via negative selection. Isolated PBMCs are suitable for flow cytometry, molecular assays, activation and expansion, cell culture, or other functional studies.

Name	Format	Quantity	Storage
BACS™ Streptavidin Microbubbles	In buffer	20 mL	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
Microbubble Leukopak Human PBMC Biotin Antibody Cocktail Microbubble	Monoclonal antibodies in PBS with <0.09% sodium azide	2 mL	2-8 °C

Additional Supplies:

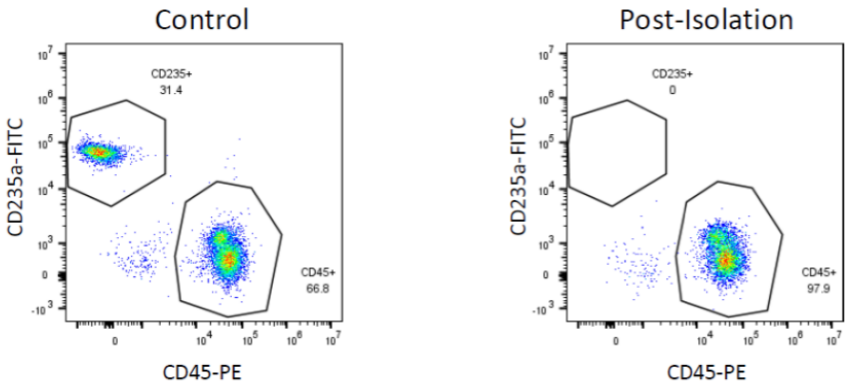
1. 20 rpm tube rotator for mixing (e.g., Thermo Scientific cat#: 88881002)
2. Centrifuge (swinging bucket rotor strongly preferred)
3. Vacuum aspirator
4. 50 mL centrifuge tubes

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
- This protocol is designed for starting samples containing 1 x 10^7 – 5.5 x 10^8 total cells, white blood cells (WBC) and red blood cells (RBC) combined. For samples outside of this range, please contact techsupport@akadeum.com

Human Leukopak PBMC Isolation:

Untouched human PBMCs were isolated from washed leukopak material. Isolated PBMCs were labeled with CD45 and CD235a. The fluorescently labeled cells were analyzed by flow cytometry. Debris and dead cells were excluded from analysis.



Experimental Setup:

Sample Size* (Before Beginning)	Initial Sample Volume (Step 9)	Antibody Cocktail (Step 11)	BACS™ Microbubbles (Step 13)	Final Volume (Step 14)
$1x10^7$–$1.65x10^8$ total cells	$3.33x10^8$ cells/mL	10 µL/$1x10^7$ cells	50 µL/$1x10^7$ cells	Fill to 1.5 mL with Separation Buffer
$1.65x10^8$–$5.5x10^8$ total cells	$3.33x10^8$ cells/mL	10 µL/$1x10^7$ cells	50 µL/$1x10^7$ cells	Fill to 5.0 mL with Separation Buffer

Prepare Sample (Remove Platelets):

Note: A platelet-removing protocol on a cell washing instrument may be used in place of steps 1 – 7.
Note: The following steps can be performed in smaller vessels (5-15 mL tubes) if starting with <10 mL raw leukopak material. Contact techsupport@akadeum.com for specific volume recommendations.

1. Aliquot up to 25 mL of leukopak material in 50 mL conical tubes.
2. Bring tubes to 25 mL with Separation Buffer and centrifuge at 400 x g for 10 min (with brake, at room temperature or 4°C).
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 25 mL with Separation Buffer and centrifuge at 400 x g for 10 min (with brake, at room temperature or 4°C).
6. Remove supernatant and resuspend each cell pellet in 1 mL of Separation Buffer.
7. Combine all cell suspensions into 1 tube.
   Note: It is recommended to wash remaining tubes with 1 mL of separation buffer and combine with cell suspension to prevent loss of cells.
8. Count total cells (WBC + RBC).
   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.
10. Transfer 1 x 10^7 – 5.5 x 10^8 cells (30 µL – 1.65 mL of cell suspension) to appropriate vessel (see Table 1).
    Note: It is essential to review the product insert regarding how to use the Microbubble Separation Tube.

Label Cells

11. Add 10 µL of Microbubble Leukopak Human PBMC Biotin Antibody Cocktail per 1 x 10^7 total cells (WBC + RBC) as indicated in Table 1. 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 a homogeneous white suspension is created.
13. Add 50 µL of BACS™ Streptavidin Microbubbles per 1 x 10^7 total cells (WBC + RBC) as indicated in Table 1.
14. Add Separation Buffer to achieve a final volume as indicated in Table 1. See Table 2 for specific examples temperature.
15. Mix samples on a rotator at 20 rpm for 10 min at room temperature.

Separate Cells:

16. Centrifuge samples at 400 x g for 5 min (with brake, at room temperature or 4°C).
    Note: A swinging bucket rotor centrifuge is recommended.
17. Vacuum aspirate the microbubbles and supernatant, taking care not to disturb the cell pellet containing the purified T cells.
18. Resuspend cell pellet in desired buffer or media for downstream use.
    Note: Transfer PBMCs to a new tube if desired.

Example Sample Compositions

Vessel Size	Sample Size Total Cells	Sample Volume (µL)	Antibody Cocktail Volume (µL)	BACSTM Microbubbles (µL)	Additional Separation Buffer (µL)	Final Microbubble Mixing Volume (µL)
1.5 mL	$1x10^7$	30	10	50	1410	1500
1.5 mL	$1.65x10^8$	495	165	825	15	1500
5.0 mL	$2.0x10^8$	600	200	1000	3200	5000
5.0 mL	$5.5x10^8$	1650	550	2750	50	5000

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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