Human Red Blood Cell Depletion Microbubbles (Cat.13210-140)

BACS™ RBC Depletion Microbubbles Protocol

Deplete human red blood cells (RBCs) from dissociated tissues or residual RBCs from blood derived samples that have previously been subjected to density gradient separation or lysis via Buoyancy Activated Cell Sorting (BACS). RBCs are targeted and removed with an antibody recognizing Glycophorin A (CD235ab). RBC-depleted cells can be used in applications such as flow cytometry, molecular assays, cell culture, and other functional studies. For depletion of up to 50 x 10^6 RBCs.

Name	Format	Quantity	Storage
BACS™ RBC Depletion Microbubbles	In buffer with 0.09% sodium azide.	1 mL	2-8 °C
Separation Buffer	Ca^{2+} and Mg^{2+} —free PBS containing 2 mM EDTA and 0.5% biotin-free BSA.	50 mL*	2-8 °C

* One 50 mL bottle of Separation Buffer will be shipped for every 5 kits ordered.

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)

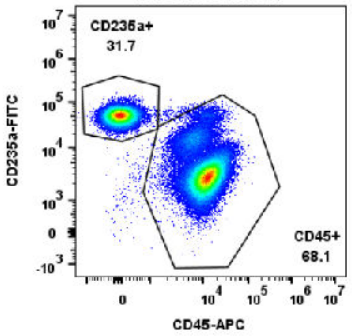
Before You Begin:

- This protocol is designed for the depletion of RBCs from mixed cell populations with up to 50-70% RBC contamination. If starting from whole blood, a density gradient separation or RBC lysis is required.
- 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 – 16 x 10^7 total cells. Samples with > 16 x 10^7 should be divided across multiple tubes. For samples <1x10^7, please contact techsupport@akadeum.com

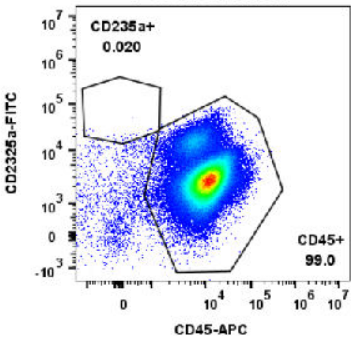
Representative RBC Depletion:

RBCs were depleted from a mixture of RBCs and white blood cells (WBCs). RBCs were labeled with CD235a-FITC and WBCs with CD45-APC. The fluorescently labeled cells were analyzed by flow cytometry. Debris and dead cells were excluded from analysis.

Pre-Isolation:
32% RBCs



Post-Isolation:
0.02% RBCs



Experimental Setup:

Sample Size	Tube Size	Sample Volume (Step 2)	BACS™ RBC Depletion Microbubbles (Step 5)	Final Volume (Step 6)
($1x10^7$ cells)		per ($1x10^7$ cells)	per ($1x10^7$ cells)	Separation Buffer
1 - 4.5	1.5 mL	40 µL	150 µL	Fill to 1.2 mL
> 4.5 - 16	5.0 mL	40 µL	150 µL	Fill to 4.0 mL

Prepare Cells:

1. Count and wash cells.
2. Resuspend cell pellet in 40 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.

Bind BACS™ RBC Depletion Microbubbles:

4. Resuspend BACS™ RBC Depletion Microbubbles by pipetting or inverting by hand.

   Note: It is critical that BACS™ RBC Depletion 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.

5. Add 150 uL of BACS™ RBC Depletion Microbubbles per 1 x 10^7 total cells to the labeled sample as indicated in the table above.
6. Add Separation Buffer to achieve a final volume of 1.2 or 4.0 mL, as indicated in the table above.
7. Mix samples on a rotator at 20 rpm for 10 min at room temperature (or at 4˚C).

Separate Cells:

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

   Note: A swinging bucket rotor centrifuge is recommended.

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

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

10. 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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