Human Red Blood Cell Depletion Kit

with Akadeum BACS™ Technology

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Components of Human Red Blood Cell Depletion Kit (# 13210-140):

IN THE BOX:

1. Human RBC Depletion Microbubbles

PACKAGED SEPARATELY:

1. Separation Buffer Ca^{2+} and Mg^{2+} free PBS containing 2 mM EDTA, 0.5% biotin-free BSA, and 0.09% sodium azide

Additional Recommended Supplies:

1. Low retention wide orifice 200 µL pipet tips
   VWR Part #37001-186 or similar
2. Centrifuge with swinging bucket rotor
3. Vacuum aspirator

PRODUCT OVERVIEW

- Akadeum's Human Red Blood Cell Depletion Microbubbles are designed to clean up samples with residual RBC contamination after RBC lysis or Ficoll™ isolation of human whole blood samples.
- Microbubbles employ a monoclonal murine IgG against the human RBC glycophorin-A sialoglycoprotein (CD235a). CD235a's high specificity for erythrocytes, and its high density on RBC surfaces make it an excellent targeting molecule for capture.
- Upon engaging undesired RBCs, Akadeum microbubbles rapidly lift these cells to the top of the sample for easy removal.
- **Note:** The exact volume of Separation Buffer included in each shipment is dependent on the number of hRBC Microbubbles ordered.

Storage: Store microbubbles and Separation Buffer at 4°C

Cell Type: Red Blood Cell

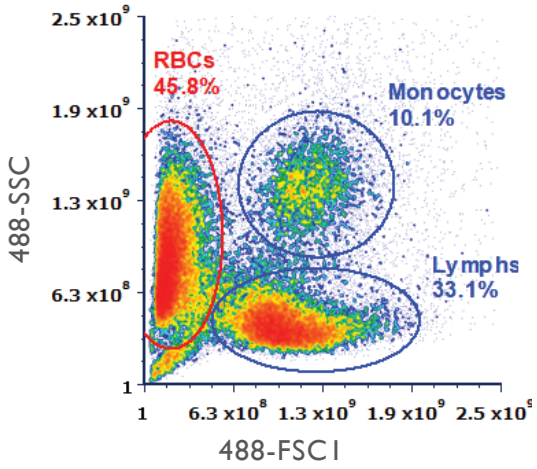
Sample Species and Source: Human whole blood or bone marrow aspirate

Cell Separation Method: Depletion

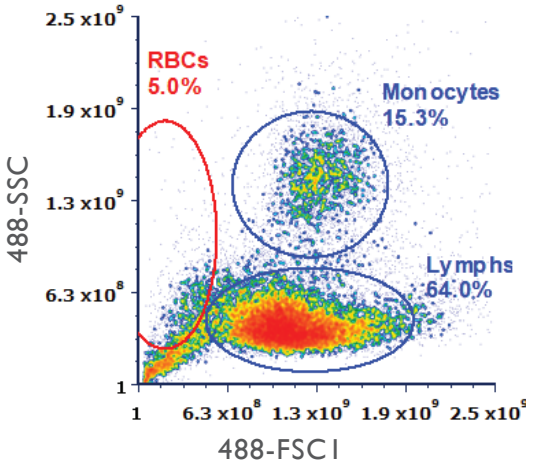
Capacity: Enough microbubbles to remove approximately 50 million RBCs

EXAMPLE OF HUMAN RED BLOOD CELL DEPLETION

Before Human Red Blood Cell Depletion



After Human Red Blood Cell Depletion



SAFETY INFORMATION

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Before use, please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

GENERAL NOTES

For additional technology or product information please visit www.akadeum.com

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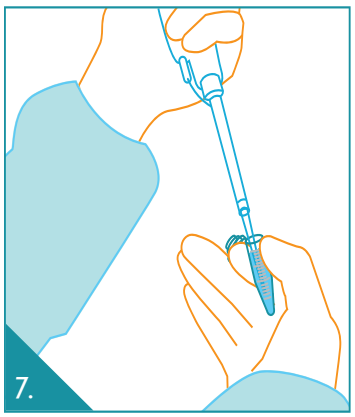
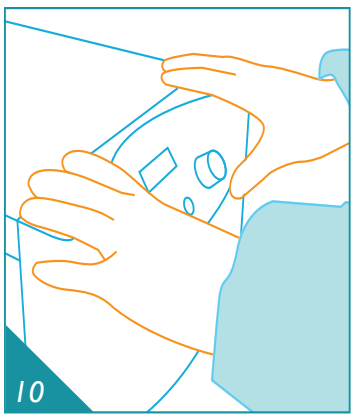
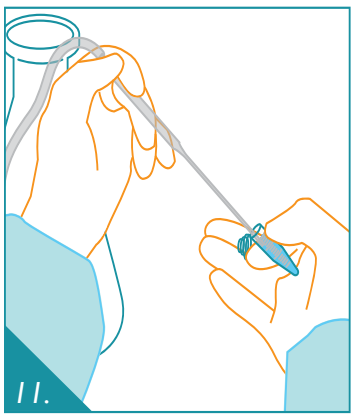
Protocol: Human Red Blood Cell Depletion

Note: *This kit is used to clean up RBC contamination after RBC lysis or Ficoll isolation of human whole blood samples.*







PREPARE YOUR CELLS

1. Determine the total cell number by counting with a manual hemocytometer or automated cell counter.
2. Centrifuge cell suspension (5 min, RT, 400 x g), aspirate supernatant, and wash once with equal volume of Separation Buffer.
3. Resuspend cell pellet in 50 µL of separation buffer per 10^7 total cells.

BIND MICROBUBBLES

4. Prepare microbubbles by resuspending microbubbles in solution (mixture should be a homogeneous white solution, i.e. look like milk). Vigorously mix or pipet. Immediately proceed to Step 5.
5. Add 150 µL of Human RBC Depletion Microbubbles per 10^7 total cells.

   Note: This volume may need to be adjusted by sample type or amount of RBC contamination.
6. Set the pipet volume to ~70% of the total sample volume (cell suspension + microbubbles).

   Note: This pipet setting ensures adequate mixing of microbubbles and cells for binding.
7. Mix microbubbles with cells by gentle trituration for 30 pipet strokes (approximately 30-60 seconds) using low retention wide orifice 200 µL pipet tip
8. Immediately add 1 mL Separation Buffer to the sample.

   Note: This facilitates separation of microbubbles from the unbound cells.
9. Repeat steps 4-8 for remaining samples making sure to resuspend microbubbles before each sample.

SEPARATE CELLS

10. Centrifuge samples (5 min, RT, 400 x g).

    Note: Use of a swinging bucket rotor for this step facilitates microbubble aspiration.
11. Aspirate off white microbubble layer and supernatant. Take care not to aspirate cell pellets.
12. Resuspend cell pellet in the desired amount of separation buffer or other buffer/media by pipetting and transfer cells to the appropriate tube/plate for use.

Table 1. Example Volumes For Cell Depletion

Total Cells	Resuspension Volume	Microbubbles	Mixing Volume
1 x 10^7	50µL	150µL	100µL
5 x 10^7	250µL	750µL	500µL

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