

## ab322034 – Human Red Blood Cell Depletion Microbubbles

For cell-based quantitative measurement of Human Red Blood Cells. 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. 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  $50 \times 10^6$  RBCs  
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab322034>

### Storage and Stability:

The entire kit can be stored at 4°C from the date of shipment. For prepared reagent storage, see table below.

### Materials Supplied

Item	Quantity	Storage Condition	Format
BACSTM RBC Depletion Microbubbles	1 mL	4°C	In buffer with 0.09% sodium azide.
Separation Buffer	50 mL*	4°C	Ca <sup>2+</sup> and Mg <sup>2+</sup> —free PBS containing 2 mM EDTA and 0.5% biotin-free BSA.

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

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 20 rpm tube rotator for mixing
- Centrifuge (swinging bucket rotor strongly preferred)
- Vacuum aspirator
- 30 µm cell strainer (optional)

### Before Starting:

- 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.
- For optimal results, homogenize mouse spleens and lyse red blood cells in the sample prior to separation.
- 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.
- This protocol is designed for starting samples containing  $1 \times 10^7$  –  $16 \times 10^7$  total cells. Samples with  $> 16 \times 10^7$  should be divided across multiple tubes.

### Experimental Setup:

Sample Size	Tube Size	Sample Volume (Step 2)	BACSTM RBC Depletion Microbubbles (Step 5)	Final Volume (Step 6)
( $1 \times 10^7$ cells)	-	per ( $1 \times 10^7$ cells)	per ( $1 \times 10^7$ 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  $1 \times 10^7$  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 BACSTM RBC Depletion Microbubbles:

4. Resuspend BACSTM RBC Depletion Microbubbles by pipetting or inverting by hand.  
*Note: It is critical that BACSTM 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 µL of BACSTM RBC Depletion Microbubbles per  $1 \times 10^7$  total cells to the labelled 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 BACSTM RBC Depletion Microbubble layer and supernatant, taking care not to disturb the cell pellet. Once BACSTM RBC Depletion Microbubbles have been aspirated, the supernatant may be removed by pipette.
10. Resuspend cell pellet in desired buffer or media and transfer to clean tube.

For technical support contact information, visit: [www.abcam.com/contactus](http://www.abcam.com/contactus)

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Version 1 | 2024-09-11