

ab322031 – Human B Cell Isolation Kit

For cell-based quantitative measurement of Human B Cells. Isolate untouched B cells from human mononuclear cells (MNCs) via Buoyancy Activated Cell Sorting (BACS). This kit can be used to target and remove non-B cells with antibodies recognizing CD2, CD3, CD14, CD15, CD16, CD36, CD43, CD56, CD57, CD123, CD161, CD235ab, and CD314. Isolated B cells are suitable for flow cytometry, molecular assays, cell culture, and other functional studies.

Processing capacity 1 x 10⁹ MNCs.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab322031>

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
BACS™ Streptavidin Microbubbles	15.5 mL	4°C	In buffer with 0.09% sodium azide.
Human B Cell Biotin Antibody Cocktail	1050 µL	4°C	Monoclonal antibodies in PBS with sodium azide.
Separation Buffer	200 mL	4°C	Ca ²⁺ and Mg ²⁺ —free PBS containing 2 mM EDTA and 0.5% biotin-free BSA.
5 mL Tubes	20 vials	4°C	Bag of tubes

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 has been optimized for MNCs as the starting material. If starting from whole blood, prepare an MNC suspension via density gradient separation.
 - For optimal results, work with fresh MNCs. If starting from previously frozen MNCs, it may be beneficial to perform a DNase I treatment 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 x 10⁷ – 16 x 10⁷ total cells. Samples with >16 x 10⁷ should be divided across multiple tubes.

Experimental Setup:

Sample Size	Tube Size	Sample Volume (Step 2)	Antibody Cocktail (Step 4)	BACS™ Microbubbles (Step 6)	Final Volume (Step 7)
(1x10 ⁷ cells)	-	per (1x10 ⁷ cells)	per (1x10 ⁷ cells)	per (1x10 ⁷ cells)	Separation Buffer
1 – 4.5	1.5 mL	30 µL	10 µL	150 µL	Fill to 1.2 mL
> 4.5 - 16	5.0 mL	30 µL	10 µL	150 µL	Fill to 4.0 mL

Prepare Cells:

1. Count and wash cells.
2. Resuspend cell pellet in 30 µL of Separation Buffer per 1 x 10⁷ 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.

Label Cells:

4. Add 10 µL of Human B Cell Biotin Antibody Cocktail per 1 x 10⁷ total cells as indicated in the table above. Gently mix samples and incubate for 10 min at room temperature (or at 4°C)

Bind BACS™ Microbubbles:

5. Resuspend BACS™ Microbubbles by pipetting or inverting by hand.
Note: It is critical that BACS™ 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.
6. Add 150 µL of BACS™ Microbubbles per 1 x 10⁷ total cells to the labelled sample as indicated in the table above.
7. Add Separation Buffer to achieve a final volume of 1.2 or 4.0 mL, as indicated in the table above.
8. Mix samples on a rotator at 20 rpm for 10 min at room temperature (or at 4°C).

Separate Cells:

9. Centrifuge samples at 400 x g for 5 min.
Note: A swinging bucket rotor centrifuge is recommended.
10. Vacuum aspirate the BACS™ Microbubble layer and supernatant, taking care not to disturb the cell pellet. Once BACS™ Microbubbles have been aspirated, the supernatant may be removed by pipette.
11. Resuspend cell pellet in desired buffer or media and transfer to clean tube.

For technical support contact information, visit: www.abcam.com/contactus