Human CD14+ Monocyte Isolation Kit (Cat.13210-220)

BACS™ Microbubbles Protocol

Isolate untouched CD14+ Monocytes from human mononuclear cells (MNCs) via Buoyancy Activated Cell Sorting (BACS). This kit can be used to target and remove non-CD14+ monocyte cells with antibodies recognizing CD3, CD5, CD15, CD16, CD19, CD20, CD56, CD57, CD66b, CD123, CD235ab, and CD314. Isolated CD14+ monocyte cells are suitable for flow cytometry, molecular assays, cell culture, and other functional studies. Processing capacity 1 x 10^9 MNCs.

Name	Format	Quantity	Storage
BACS™ Streptavidin Microbubbles	In buffer with 0.09% sodium azide.	15.5 mL	2-8 °C
Human CD14+ Monocyte Biotin Antibody Cocktail	Monoclonal antibodies in PBS with sodium azide.	1050 µL	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
5 mL Tubes	Bag of tubes	20 tubes	RT

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)

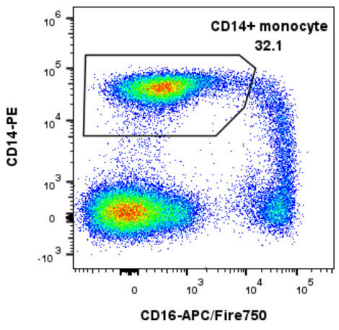
Before You Begin:

- This protocol has been optimized for MNCs as the starting material. If starting from whole blood, prepare an MNC suspension via density gradient separation. If working with other sample types, please contact techsupport@akadeum.com.
- 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.
- 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

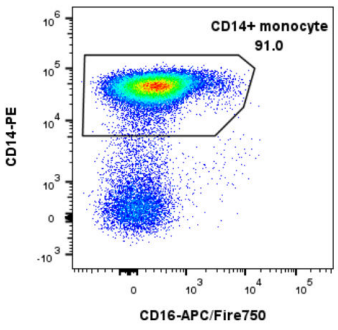
Representative CD14+ Monocyte Isolation:

Untouched CD14+ monocytes were isolated from MNCs. Isolated cells were labeled with CD14-PE and CD16-APC/Fire750. The fluorescently labeled cells were analyzed by flow cytometry. Debris and dead cells were excluded from analysis.

Pre-Isolation:
32% CD14+ Monocyte



Post-Isolation:
91% CD14+ Monocyte



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 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.

Label Cells:

4. Add 10 µL of Human CD14+ Monocyte Isolation Biotin Antibody Cocktail per 1 x 10^7 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 uL of BACS™ Microbubbles per 1 x 10^7 total cells to the labeled 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.

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

11. 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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