

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

BACS™ Microbubbles Protocol

Isolate untouched CD14+ Monocytes from human Peripheral Blood Mononuclear Cells (PBMCs) 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 10⁹ PBMCs.

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:

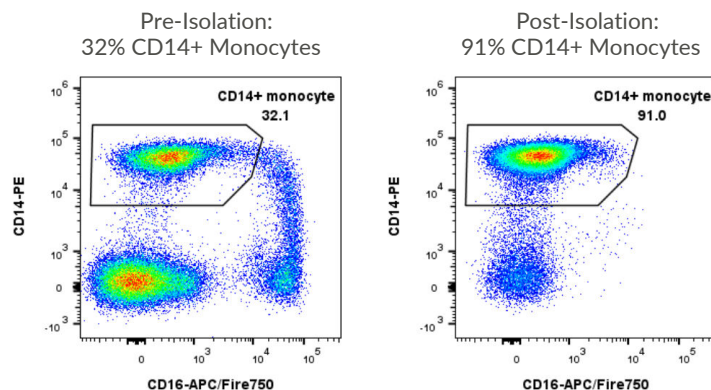
- 1 20 rpm tube rotator for mixing (e.g., Thermo Scientific cat#: 88881002)
- 2 Centrifuge (swinging bucket rotor strongly preferred)
- 3 Vacuum aspirator
- 4 30 µm cell strainer (optional)

Before You Begin:

- ▶ For best results, we strongly recommend [watching our video](#) about how to work with microbubbles, particularly if you haven't worked with microbubbles previously.
- ▶ For optimal results, work with fresh PBMCs. If starting with previously frozen PBMCs, it may be beneficial to perform a DNase treatment prior to separation. If working with other sample types, please contact techsupport@akadeum.com.
- ▶ 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 10⁷ – 1.6 x 10⁹ total cells. Samples with > 1.6 x 10⁹ should be divided across multiple tubes. For samples <1x10⁷, please contact techsupport@akadeum.com

Representative CD14+ Monocyte Isolation:

Untouched CD14+ monocytes were isolated from PBMCs. 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.



Experimental Setup:

Sample Size	Tube Size	Sample Volume (Step 2)	Antibody Cocktail (Step 4)	BACSTM 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:

- Count and wash cells.
- Resuspend cell pellet in 30 µL of Separation Buffer per 1 x 10⁷ cells, as indicated in the table above.
- 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 and dispense into the appropriate number of tubes.
- Add 150 µL of BACSTM Microbubbles per 1 x 10⁷ total cells to the labeled sample as indicated in the table above.
- Add Separation Buffer to achieve a final volume of 1.2 or 4.0 mL, as indicated in the table above.
- Mix samples on a rotator at 20 rpm for 10 min at room temperature.

Label Cells:

- Add 10 µL of Human CD14+ Monocyte Isolation Biotin Antibody Cocktail per 1 x 10⁷ total cells as indicated in the table above. Gently mix samples and incubate for 10 minutes at room temperature.

Bind BACSTM Microbubbles:

- Resuspend BACSTM Microbubbles by pipetting or inverting by hand.

Note: It is critical that BACSTM 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.

Separate Cells:

- Centrifuge samples at 400 x g for 5 min with the brake on.

Note: A swinging bucket rotor centrifuge is recommended.

- Vacuum aspirate the BACSTM Microbubble layer and supernatant, taking care not to disturb the cell pellet. Once BACSTM Microbubbles have been aspirated, the supernatant may be removed by pipette. For the best results, we strongly recommend [watching our video about How To Remove BACSTM Microbubbles](#).



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

- Resuspend cell pellet in desired buffer or media and transfer to clean tube.



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