



# Human CD4+ T Cell Isolation Kit (Cat.13210-130)

(Cayman Item No. 44562)

## BACS™ Microbubbles Protocol

Isolate untouched CD4+ T cells from human mononuclear cells (MNCs) via Buoyancy Activated Cell Sorting (BACS). This kit can be used to target and remove non-CD4+ T cells with antibodies recognizing CD8, CD11b, CD14, CD16, CD19, CD20, CD36, CD56, CD123, CD161, CD235ab, CD314, and  $\gamma\delta$ TCR. Isolated CD4+ T cells are suitable for flow cytometry, molecular assays, activation and expansion, cell culture, and other functional studies. Processing capacity 1 x 10<sup>9</sup> MNCs.

Name	Format	Quantity	Storage
BACS™ Streptavidin Microbubbles	In buffer with 0.09% sodium azide.	15.5 mL	2-8 °C
Human CD4+ T Cell Biotin Antibody Cocktail	Monoclonal antibodies in PBS with sodium	1050 $\mu$ L	2-8 °C
Separation Buffer	Ca <sup>2+</sup> and Mg <sup>2+</sup> –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:

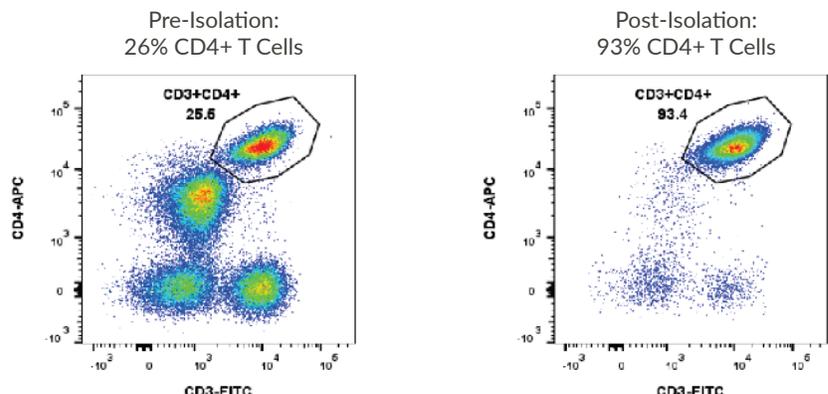
- 20 rpm tube rotator for mixing (e.g., Thermo Scientific cat#: 88881002)
- Centrifuge (swinging bucket rotor strongly preferred)
- Vacuum aspirator
- 30  $\mu$ m cell strainer (optional)

### 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  $\mu$ m 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<sup>7</sup> – 16 x 10<sup>7</sup> total cells. Samples with > 16 x 10<sup>7</sup> should be divided across multiple tubes. For samples < 1x10<sup>7</sup>, please contact techsupport@akadeum.com

### Representative CD4+ T Cell Isolation:

Untouched CD4+ T cells were isolated from MNCs. Isolated cells were labeled with CD3-FITC and CD4-APC. 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 <sup>7</sup> cells)		per (1x10 <sup>7</sup> cells)	per (1x10 <sup>7</sup> cells)	per (1x10 <sup>7</sup> 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 10<sup>7</sup> 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.
- Add 150 µL of BACSTM Microbubbles per 1 x 10<sup>7</sup> 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 (or at 4°C).

## Label Cells:

- Add 10 µL of Human CD4+ T Cell Isolation Biotin Antibody Cocktail per 1 x 10<sup>7</sup> total cells as indicated in the table above. Gently mix samples and incubate for 10 min at room temperature (or at 4°C).

## 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.  
*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.



*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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