ab322029 – Mouse Naïve CD4+ T Cell Isolation Kit

For cell-based quantitative measurement of Mouse Naïve CD4+ T Cells. Isolate untouched Naïve CD4+ T cells from mouse splenocytes via Buoyancy Activated Cell Sorting (BACS). This kit can be used to target and remove non-naïve CD4+ T cells with antibodies recognizing CD8a, CD11b, CD11c, CD19, CD24, CD25, CD44, CD49b, CD105, Gr1, Ter119, and TCRγδ. Isolated naïve CD4+ T cells are suitable for flow cytometry, molecular assays, activation and expansion, cell culture, and other functional studies. Processing capacity 1 x 10^9 cells.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab322029

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	10.5 mL	4°C	In buffer with 0.09% sodium azide.
Mouse Naïve CD4+ T 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 splenocytes as the starting material.
- 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 x 10^7 – 24 x 10^7 total cells. Samples with > 24 x 10^7 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 - 7	1.5 mL	30 µL	10 µL	100 µL	Fill to 1.2 mL
> 7 - 24	5.0 mL	30 µL	10 µL	100 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 Mouse Naïve CD4+ T Cell 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 100 µL of BACS™ Microbubbles per 1 x 10^7 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

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