XpresSep™ Human CD4 Negative Selection Kit

Catalog #: HCD4-N109 Capacity: 1 x 10^9 cells

1. Product Description

Materials

Supplied	• 0.5mL Monoclonal Antibody Cocktail
	• 0.5mL XpresSep™ Ferrofluid
	• 10mL Labeling/Separation buffer (10x concentrated)
Required	• ddH_2O
	• XpresSep™ Multipole Magnetic Separator
Storage and Stability	Materials may be shipped at room temperature, but should be stored protected from light at 4–8°C upon receipt. Do not freeze. The expiration date is indicated on the vial label.

2. Product applications

Negative Selection of $CD4^+$ cells from apheresis, PBMC, or cell culture suspensions. The untouched, isolated cells can be used for phenotype studies, functional assays, and culture expansion.

3. Protocol

The volumes stated here are for separation of $5x10^7$ cells, scale up or down as needed.

I. Reagent Preparation: prepare before each experiment.

a. Labeling/Separation Buffer: dilute 10x buffer with ddH_2O to 1x, the 1x buffer is referred to as buffer throughout protocol, store at 2-8˚C when not in use.

b. Antibody Cocktail: prepare 250 µL per $5x10^7$ cells by adding 25 µL of antibody cocktail to 225 µL of buffer, keep the diluted antibody solution on ice prior to use.

c. Ferrofluid preparation: prepare 0.5 mL per $5x10^7$ cells, dilute 24 µL of Ferrofluid in 476 µL of buffer and mix, and keep on ice prior to use.

II. Cell Preparation

a. Prepare a single-cell suspension. If working with whole blood, perform a PBMC fraction isolation via the Ficoll-Paque method. Wash and re-suspend cell pellet in buffer.

b. Count cells and adjust the cell concentration to $2x10^8$ cells/mL with buffer.

III. Antibody/Ferrofluid Labeling

a. For every $5x10^7$ cells, add 250 µL of diluted antibody to a 12x75 tube, then add 250 µL of cell suspension on top of the antibody and mix gently by pipetting up and down once.

Note: Do not vortex as this could damage cells.

b. Incubate at room temperature for 15 min.

c. Add 0.5 mL of Ferrofluid to the cell suspension and mix by inversion.

d. Incubate for 10 min at room temperature.

IV. Cell Separation

a. After incubation, add 1.5 mL of buffer to the tube and mix well by gentle pipetting.

b. Place the tube with cell mixture in the appropriate XpresSep™ multipole separator.

i. 1 mL – 4 mL → 12x75 tube: XpresSep™5

ii. >4 mL – 14 mL → 15 mL tube: XpresSep™15

iii. >14 mL – 45 mL → 50 mL tube: XpresSep™50

c. Incubate for 10 min.

d. Keep the tube in the separator and aspirate the supernatant with a long Pasteur pipette, making sure not to touch the sides of the tube. Alternatively, you can pour the supernatant into another fresh tube. The supernatant contains the untouched target cells.

e. The provided buffer can be used for fluorescent labeling and flow analysis.

Safety

The Ferrofluid contains 0.05% Proclin™ 300. This concentration presents no health hazards, toxicology problems, or disposal issues. Please consult the Safety Data Sheet for additional information.

Products are for **RESEARCH USE ONLY** and are not intended for human or animal therapeutic or diagnostic uses.

Warranty

The products are warrantied only against defects in workmanship and quality at the time of delivery. BioMagnetic Solutions LLC makes no warranty beyond the technical specifications of the product. BioMagnetic Solutions LLC liability is limited to either replacement of the products or refund of the purchase price.

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