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CytoSinct™ CD4 Nanobeads, human
Cat.No. L00863

Contents

I. Product Description

The CytoSinct™ CD4 Nanobeads, human are used for separating CD4+ cells from fresh or frozen peripheral blood mononuclear cells (PBMCs), leukapheresis products or single cell suspension based on the surface expression of human CD4. The surface of Nanobeads are labeled with anti-human CD4 monoclonal antibody. To begin the isolation, Nanobeads are added to the cells. The CD4 antibodies preconjugated to the Nanobeads can bind the target cells expressing CD4 on cell surface. The cells/beads mixed suspension is loaded onto a CytoSinct™ Column which is effectively magnetized by an external magnetic field from CytoSinct™ Magnet, or other compatible cell isolation columns and magnets. The Nanobeads-labeled CD4+ cells are retained within the column and enriched during the wash step when Isolation Buffer is used to flush out the CD4- cells. After removing magnetic field, the target CD4+ cells can be easily eluted from the column.

II. Product Specification

Cat. No.	Name	Size	Capacity
L00863-0.5	CytoSinct™ CD4 Nanobeads, human	500 µL	for up to 5×10^8 total cells
L00863-1	CytoSinct™ CD4 Nanobeads, human	1 mL	for up to 1×10^9 total cells

Reactivity	Human
Product format	Bio-degradable matrix coated nanoparticle conjugated with anti-CD4 antibodies supplied in phosphate buffered-saline (PBS), containing Human Serum Albumin (HSA), pH 7.0-7.4.
Application	Positive selection or depletion of CD4+ T cells from leukapheresis, PBMC or cell cultures. Isolated CD4+ T cells can be used for culture and

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	expansion, flow cytometry, T cells functional assays.
Storage	Store at 2-8 °C. Do not freeze.

III. Requirement Materials

1. Isolation Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA) and 2 mM EDTA .

- Keep Isolation Buffer cold (2−8°C).
- BSA can be replaced by human serum albumin (HSA), human serum or fetal bovine serum (FBS).
- EDTA can be replaced by sodium citrate.
- PBS containing Ca^{2+} or Mg^{2+} is not recommended.

2. Columns and separators:

- For samples containing less than 2×10^8 total MNCs or less than 10^7 labeled cells, use CytoSinct™ gM Column and CytoSinct™ M1 Magnet or CytoSinct™ M8 Magnet, or other compatible columns and magnets.
- For samples containing less than 2×10^9 total cells or less than 10^8 labeled cells, use CytoSinct™ gL Column and CytoSinct™ L1 Magnet or CytoSinct™ L4 Magnet or other compatible columns and magnets.

IV. Protocol

All procedures are to be performed at room temperature unless otherwise instructed in this protocol.

1. Prepare Nanobeads

Gently mix the Nanobeads by pipetting for several times.

2. Prepare samples

2.1 Prepare PBMCs.

- When working with anticoagulated peripheral blood, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation method (for example, using Ficoll-Paque™ PLUS density gradient media) and washed by Isolation Buffer to remove interfering factors.
- When working with frozen PBMCs, resuscitate frozen PBMCs and then proceed with the protocol. When dead cells are found to be considerable, apply density gradient centrifugation method (for example, using Ficoll-Paque™ PLUS density gradient media) to remove dead cells, or culture cells in medium overnight before proceeding with this protocol.

2.2 Centrifuge PBMCs at 300×g for 10 minutes at room temperature (15 - 25°C). Aspirate the supernatant completely. Determine cell number by using a hemocytometer or other suitable

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methods.

3. Magnetic labeling

3.1 Transfer desired number of cells into a new tube and resuspend into single cell suspension at 10^8 mononuclear cells (MNCs) per 1 mL in Isolation Buffer.

- When working with less than 10^7 MNCs, use Isolation Buffer volume of 100 µL.

3.2 Add 10 µL Nanobeads for each 100 µL cell suspension of 10^7 total MNCs.

- When working with higher number of cells, scale up the volume of Nanobeads accordingly (e.g. for 2×10^7 total MNCs，use 20 µL Nanobeads.)

- When working with less than 10^7 MNCs, use the same Nanobeads volume of 10 µL, as that in 10^7 MNCs.

3.3 Mix the Nanobeads and cells well by gently pipetting or tapping on the bottom of the tube, and incubate for 15 min at 2 – 8 °C.

3.4 Wash cells once by adding 1-2 mL of Isolation Buffer per 10^7 MNCs, mix well by gentle pipetting, and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

3.5 Resuspend up to 10^8 cells in 500 µL of Isolation Buffer.

- Scale up the volume of Isolation Buffer accordingly when more than 10^8 MNCs are to be processed.

4. Magnetic separation

4.1 Choose an appropriate CytoSinct™ Column and CytoSinct™ Magnet or other compatible columns and magnet according to the number of total cells and the number of CD4+ cells as instructed in Section III.

4.2 Assemble the column onto the suitable CytoSinct™ Magnet or other compatible magnet (please refer to manual of CytoSinct™ Magnet or other compatible magnets for assembly instructions).

4.3 Rinse the column once with Isolation Buffer (500 µL for CytoSinct™ gM column, 3 mL for CytoSinct™ gL Column, or other compatible columns) and let the buffer run through it but not run dry.

4.4 Transfer the cell suspension onto the prepared CytoSinct™ gM or CytoSinct™ gL Column or other compatible columns using a pipette and collect the unlabeled cells in flow-through.

4.5 Wash the column with Isolation Buffer (500 µL × 3 for CytoSinct™ gM Column, 3 mL × 3 for CytoSinct™ gL Column, or other compatible columns). Collect unlabeled cells in flow-through with a suitable tube (for example, a 2 mL or 15 mL conical tube). Repeat the washing step for another two times. Add new Isolation Buffer when the column stops dripping but not run dry.

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4.6 Remove the column from the magnet and place it on a new tube with suitable size (for example, a 15 mL or 50 mL conical tube).

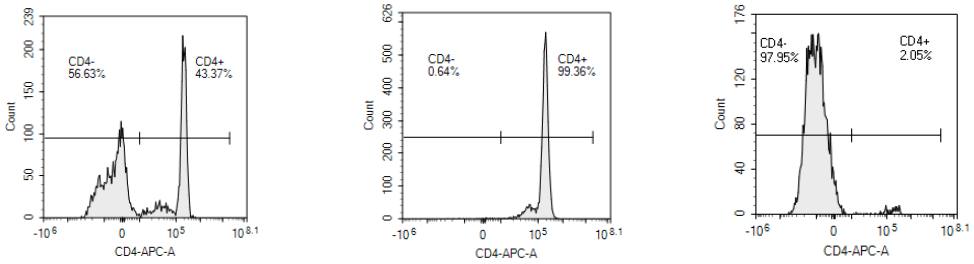
4.7 Pipette the Isolation Buffer onto the column (1 mL for CytoSinct™ gM Column, 5 mL for CytoSinct™ gL Column, or other compatible columns). Immediately flush out the fraction with the magnetically labeled cells by firmly applying the plunger through the column chamber supplied with the column.

4.8 The cells can then be counted, analyzed to assess the purity or used in down-stream applications. The Nanobeads do not need to be removed. To ensure cell viability, the desired cell fraction should be immediately resuspended in cell culture medium.

V. Product Example Data

CD4+ T cells were isolated from human PBMCs using CytoSinct™ Human CD4 Nanobeads and CytoSinct™ gM columns. CD4+ cells were stained with anti- CD4 (SK3) APC and gated on Live/hCD45+.

PBMC before separation **Isolated CD4+ T cells** **CD4+ T cell depleted fraction**



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