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CytoSinct™ CD8 Nanobeads, human

Contents

I. Product Description

CytoSinct CD8 Nanobeads, human is used for *in vitro* enrichment of $CD8^+$ cells from fresh or frozen peripheral blood mononuclear cells (PBMCs), leukapheresis products, or single-cell suspension based on the surface expression of human CD8.

To begin the isolation, Nanobeads are added to the cells. The cells/beads mixed suspension is loaded onto a CytoSinct column placed in the CytoSinct magnet, or other compatible columns with a magnet.

The $CD8^+$ cells labeled with Nanobeads are retained within the column and unlabelled cells ($CD8^-$ cells) are flushed out during the wash step. After removing the column from the magnetic field, the retained $CD8^+$ cells can be eluted as the positively selected cell fraction.

II. Product Specification

Cat. No.	Product Name	Size	Capacity
L00864-0.5	CytoSinct™ CD8 Nanobeads, human	500 µL	Up to 5×10^8 total cells
L00864-1	CytoSinct™ CD8 Nanobeads, human	1 mL	Up to 1×10^9 total cells

Components 1 mL or 0.5 mL CytoSinct CD8 Nanobeads, human
Nanobeads conjugated to monoclonal anti-human CD8 antibodies (isotype: mouse IgG1).

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Product Format	Supplied in phosphate-buffered saline (PBS), containing Human Serum Albumin (HSA) and Poloxamer 188.
Storage	Store at 2 - 8 °C. Do not freeze.

III. Required 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 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 mononuclear cells (MNCs) or less than 10^7 labeled cells, use gM columns, M1 or 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 gL columns, L1 or 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, PBMCs should be isolated by the density gradient centrifugation (DGC) and washed with Isolation Buffer to remove interfering factors.
- When working with frozen PBMCs, if dead cells are found to be considerable, a DGC is recommended to remove dead cells or culture cells in a medium overnight before proceeding.

2.2 Centrifuge PBMCs at 300 × g for 10 minutes at room temperature (15 - 25°C). Aspirate the supernatant completely and determine the cell number.

Note: for cell counting and viability determination, we recommend using **fluorescence-based**

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assays such as AO/PI instead of Trypan Blue. Trypan Blue tends to overestimate sample viability, particularly for blood samples.

3. Magnetic Labeling

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

- For less than 10^7 MNCs, use 100 µL Isolation Buffer volume.

3.2 Aadd 10 µL Nanobeads per 10^7 total MNCs. (e.g. for 2 × 10^7 total MNCs, use 20 µL Nanobeads).

- When working with less than 10^7 MNCs, use 10 µL Nanobeads as that in 10^7 MNCs (e.g. for enrichment from 5 × 10^6 total MNCs, use 10 µL Nanobeads).

3.3 Mix the Nanobeads and cells well by gently pipetting or tapping on the bottom of the tube, and incubate for 15 minutes 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 the appropriate column and magnet according to Section III.

4.2 Place the column onto the magnet.

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

4.4 Transfer the cell suspension onto the prepared gM or gL column using a pipette and collect the unlabeled cells in flow-through.

4.5 Wash the column with Isolation Buffer (500 µL × 3 for gM column, 3 mL × 3 for gL column). 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.

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 gM column, 5 mL for gL column). Immediately flush out the fraction with the magnetically labeled cells by firmly applying the plunger through the column chamber supplied with the column.

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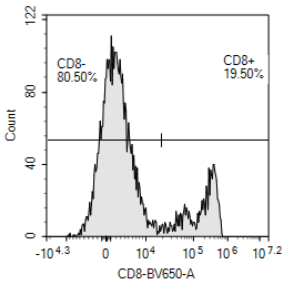
4.8 The cells can then be countsed, and analyzed to assess their purity or used in downstream applications. The Nanobeads do not need to be removed. To ensure cell viability, the desired cell fraction should be immediately resuspended in cell culture medium.

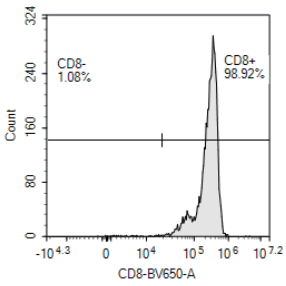
V. Representative Data

$CD8^+$ T cells were enriched from human PBMCs. $CD8^+$ cells were stained with anti-CD8 (clone: SK1) BV650 and gated on Live/$hCD45^+$.

Starting Material



Enrichment



For research use only. Not intended for human or animal clinical trials, therapeutic or diagnostic use.

Manufacturer: Nanjing GenScript Biotech Co., Ltd. No. 28 Yongxi Road, Jiangning District, Nanjing, Jiangsu, China

GenScript USA, Inc.
860 Centennial Ave. Piscataway, NJ 08854 1-732-885-9188