Cellectra™ Human CD3 NanoDeplet Microbeads, Research grade

Cat. No. MBS-S015

Product Information

Product	Size	Amount
Cellectra™ Human CD3 NanoDeplet Microbeads, Research grade	2 mL	For 10^9 total cells

Product Description

Cellectra™ Human CD3 NanoDeplet Microbeads, Research grade are dextran-coated superparamagnetic iron oxide nanoparticles of which the surface is conjugated with recombinant monoclonal antibodies specific to human CD3 (Isotype: mouse IgG1). They are especially designed for the depletion of $CD3^+$ cells.

Cellectra™ Human CD3 NanoDeplet Microbeads, Research grade are produced under sterile manufacturing conditions (ISO 5), and no animal- or human-derived components are used throughout the production process. It is produced under our rigorous quality control system that includes a comprehensive set of tests including sterility and endotoxin tests.

Product Applications

Cellectra™ Human CD3 NanoDeplet Microbeads, Research grade are designed for the depletion of $CD3^+$ cells from fresh or frozen human peripheral blood mononuclear cells (PBMCs). CD3E, also known as CD3 epsilon, is a member of the immunoglobulin superfamily found on mature T lymphocytes and most thymocytes. Depletion of T cells yields a purified non-T cell population suitable for diverse downstream applications. It supports the establishment of T cell-free systems to independently study innate immune cells such as NK cells, B cells, monocytes and dendritic cells for functional assays, single-cell sequencing and drug screening, eliminating experimental interference caused by T cells. The magnetic Microbeads are conjugated with monoclonal antibodies targeting human CD3 protein, enabling $CD3^+$ cells to be labeled with the specific antibodies and magnetic particles, and separated using the separation columns and magnets.

The Product performance has been carefully validated and tested for compatibility for cell culture use or any other applications in the early preclinical stage. For use in clinical phases, we also offer a custom GMP production service that tailors to your needs. We will work with you to customize and develop a GMP-grade product in accordance with your requests that also meets the requirements for raw and ancillary materials use in cell manufacturing of cell-based therapies.

Formulation

Supplied in PBS buffer containing EDTA, rHSA and Poloxamer 188.

Storage

For long term storage, the product should be stored in liquid state at -20°C or below

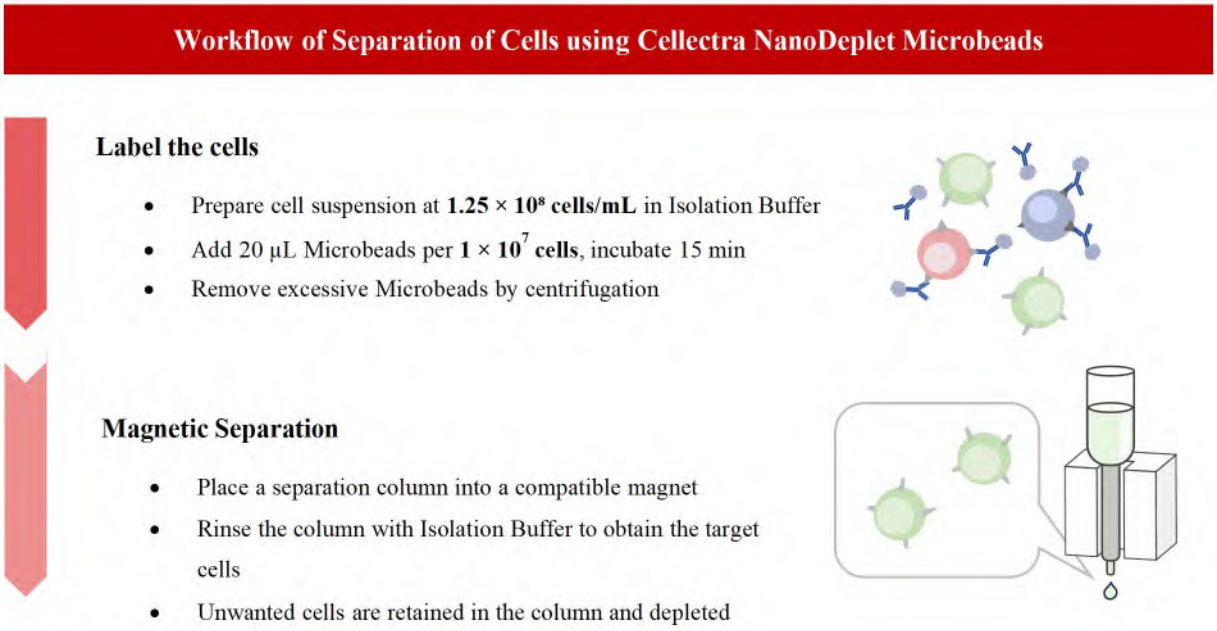
Stability upon receipt:

- Stable for 24 months at -20 °C or below;
- Stable for 6 months at 2–8 °C under sterile conditions.

Please protect from light and avoid repeated freeze-thaw cycles.

Important Note

This product is for research use only and not intended for therapeutic or diagnostic use.



General guidelines

Required Materials

- Isolation Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% BSA, and 2 mM EDTA and keep it cold (2−8°C).

  Note: BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). EDTA can be replaced by sodium citrate. PBS containing Ca^{2+} or Mg^{2+} is not recommended.
- Separation Column: MS/LS/LD separation columns or equivalent performance from other suppliers are acceptable as alternatives.
- Use LD columns if higher depletion efficiency is required, resulting in high target cell purity.
- Reduce Microbeads dosage accordingly if better recovery of target cells is desired.
- Magnetic Separators compatible to LS, MS and LD column, or equivalent performance from other suppliers are acceptable as alternatives.

PBMC Preparation

1. Prepare a single-cell suspension of human PBMCs at the concentration of 1.25 x 10^8 cells/mL in Isolation Buffer.

   Note: a. For anticoagulated blood: Isolate PBMCs using Ficoll-Paque™ density gradient centrifugation.

   b. For frozen PBMCs: Thaw and remove dead cells via Ficoll-Paque™ density gradient centrifugation.

   c. Wash all PBMCs with Isolation Buffer (300×g, 10 min, 15-25℃) before suspension preparation.

Magnetic labeling

2. Transfer the required volume of PBMC single-cell suspension (1.25×10^8 cells/mL in Isolation Buffer) into a new tube.
3. Add 20 µL Microbeads for every 10^7 PBMCs suspended in 80 µL buffer, to a total volume of 100 µL for incubation.

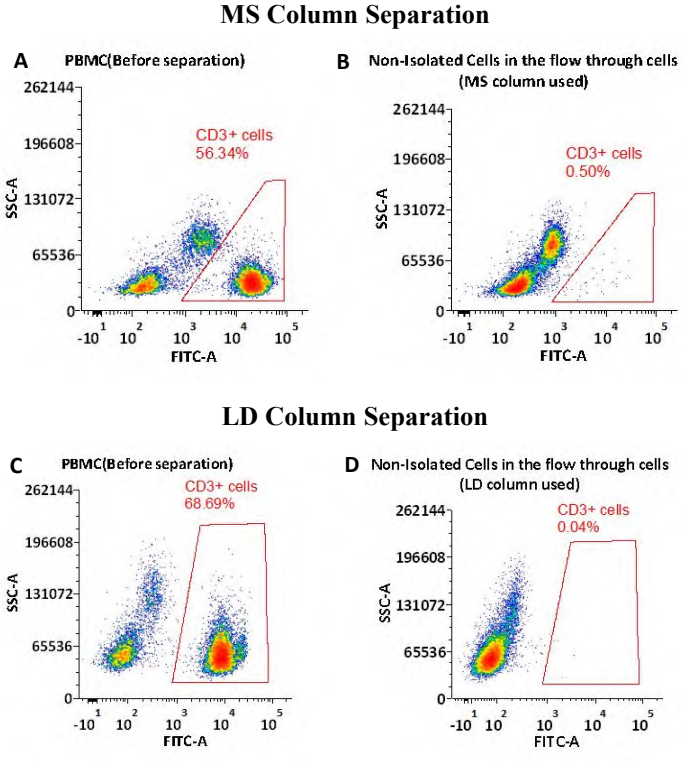
4. Mix the cell-bead mixture by gently pipetting up and down, and incubate for 15 min at 2~8 °C.
5. Add 400 µL of isolation buffer, centrifuge at 300×g for 10 minutes at room temperature, and carefully aspirate the supernatant.
6. Repeat washing ***step 5***.
7. Centrifuge at 300×g for 10 minutes, and then remove the supernatant completely.
8. Resuspend the cells in 500 µL Isolation Buffer per 1×10^7 cells for separation.

Magnetic separation

9. Place MS column in the magnetic field of a separator.
10. Prepare the separation column by rinsing it with 1000 µL of Isolation Buffer.
11. Apply cell suspension to primed the separation column, and collect the flow-through containing unlabeled cells.
12. Wash the separation column with 500 µL of Isolation Buffer twice, and collect the flow-through containing unlabeled cells.
13. Pool the flow-through fractions collected from ***step11*** and ***step12***, these fractions contain target cells.

    Note: To increase the purity of CD3$^-$ cells, the flow-through can be enriched over a second MS column. Repeat the magnetic separation procedure as described by using a new column.
14. Analyze the fractions to assess the purity or used in down-stream applications.

● Example of a separation



Percentage of CD3$^+$ cells pre- and post-depletion with Cellectra™ Human CD3 NanoDeplet Microbeads, Research Grade.
Human PBMCs(per 10^7 cells) were isolated by 20 µL Cellectra™ Human CD3 NanoDeplet Microbeads, Research grade (Cat. No. MBS-S015). The non-isolated cells were stained with FITC-anti human CD3 Antibody and 7-AAD, and subsequently analyzed by flow cytometry(QC tested). Figures A and B show cells before and after separation using the MS column, while Figures C and D present cells before and after separation using the LD column. As shown in the results, using LD columns leads to higher depletion efficiency, achieving higher target cell purity.

Disclaimer

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