SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based)

Product Instruction Manual

Cat. No.	GMP-SMT-219-Ab01-nanoIMB
Product Name	SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based)
Storage Temperature	2-8 °C (Do not freeze)

Product Introduction

SOLIDEX®-ISOEx anti-Biotin Nanobeads are designed for the in vitro enrichment or depletion of cells labeled with biotinylated antibodies or ligands from fresh or frozen Periperal Blood Mononuclear Cells (PBMCs), leukapheresis products, or single-cell suspensions. It is widely applicable for positive selection or depletion, including cells with low antigen expression.

Isolation Principle: First, cells are labeled with a biotinylated primary antibody or ligand, followed by the addition of SOLIDEX®-ISOEx anti-Biotin Nanobeads. The cell-Nanobead suspension is applied to a SOLIDEX®-ISOEx Column or other compatible Columns. Cells magnetically labeled with Nanobeads are retained within the Column, while unlabeled cells pass through and are collected during the washing steps. After removing the Column from the magnetic field, the retained labeled cells can be eluted as the positively selected cell fraction.

Product Components and Specifications

Product Specifications	50 Tests	100 Tests	750 Tests
SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based)	0.5mL	1mL	7.5mL

Note: This product is for research and development use only.
Shelf Life: Store at 2-8°C, protected from light, and do not freeze. Under these conditions, the product is valid for 6 months.

Reagents and Equipment Required

A. Cell isolation Column
For positive selection or depletion, recommended for use with GeneMedi **SOLIDEX®-ISOEx cell isolation columns**: M Column for standard throughput, Cat. No.: **GMP-ISOEx-Column-M**; L Column for high throughput, Cat. No.: **GMP-ISOEx-Column-L**. Comparable columns from other mainstream brands are also compatible.
B. Cell isolation buffer
Phosphate-buffered saline (PBS), pH 7.2, containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA (user-supplied).

Note:
(1) Keep the Cell isolation buffer cold (2-8°C).
(2) EDTA can be replaced by other supplements such as anticoagulants citrate-dextrose formula-A (ACD-A) or citrate-phosphate-dextrose (CPD).
(3) BSA can be replaced by other proteins such as human serum albumin (HSA), human serum, or fetal bovine serum (FBS).
(4) PBS containing Ca^{2+} or Mg^{2+} is not recommended.
(5) Degas the Cell isolation buffer before use, as air bubbles may block the Column.

C. Biotinylated antibodies, peptides, or ligands.

Protocol

A. Preparation of Nanobeads

Gently pipette the SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based) up and down several times to mix well before use.

B. Sample Preparation

When processing anticoagulated peripheral blood or buffy coats, isolate PBMCs via density gradient centrifugation. After density gradient separation, remove platelets by resuspending the cell pellet in buffer and centrifuging at 200 × g for 10-15 minutes at 20 °C. Carefully aspirate the supernatant. Repeat the washing step.

Note:

(1) When processing anticoagulated peripheral blood, PBMCs should be isolated by density gradient centrifugation (DGC) and washed with Cell isolation buffer to remove interfering factors.

(2) When processing frozen PBMCs, if a large number of dead cells are present, it is recommended to perform DGC to remove dead cells or culture the cells in medium overnight before proceeding.

C. Magnetic Labeling

Note:

(1) Work quickly, keep cells cold, and use pre-cooled solutions. This prevents capping of antibodies on the cell surface and non-specific cell labeling.

(2) The volumes indicated below are for up to 1×10^7 cells. When processing fewer than 1×10^7 cells, use the same volumes. When processing more cells, scale up all reagent volumes and total volumes accordingly (e.g., for 2E7 cells, use twice the volume of all specified reagents).

(3) For optimal results, prepare a single-cell suspension prior to magnetic labeling. Pass cells through a 30 µm nylon mesh (Pre-Separation Filters) to remove cell clumps that may clog the Column. Pre-wet the filter with Cell isolation buffer before use.

(4) The recommended incubation temperature is 2–8 °C. Higher temperatures or prolonged incubation times may lead to non-specific cell labeling. Incubation on ice may require longer incubation times.

a. Determine cell number. Centrifuge at 300 × g for 10 minutes. Aspirate the supernatant completely and resuspend the cell pellet in 50 µL of Cell isolation buffer per 1×10^7 cells.

b. Add an appropriate amount of Human or Mouse FcR blocking reagent. Refer to the specific FcR blocking reagent data sheet for instructions. (Optional)

c. Add an appropriate amount of biotinylated antibody (The amount of biotinylated antibody should be optimized; generally, 0.1 µg - 1 µg of antibody is used per 1×10^7 cells. Titration is recommended). Incubate at 4°C for 5-15 minutes.

Note: *Insufficient biotinylated antibody may lead to incomplete isolation, while excessive amounts may cause non-specific binding.*

d. Wash cells by adding 1 mL of Cell isolation buffer per 1×10^7 cells. Centrifuge at 300×g for 10 minutes. Aspirate the supernatant completely.

e. Repeat step d. (Optional)

f. Resuspend the cell pellet in 50-100 µL of Cell isolation buffer per 1×10^7 total cells.

g. Add an appropriate amount of anti-Biotin Nanobeads (typically 10 µL) per 1×10^7 total cells.

Note: *Use 5-10 µL of Nanobeads for enriching positive cells; use 10-20 µL of Nanobeads for depletion. The optimal amount is determined by the target cell type and the biotinylated antibody used.*

h. Mix well and incubate at 2-8°C (refrigerator) for 10-15 minutes.
i. Wash cells by adding 1-2 mL of Cell isolation buffer per 1×10^7 cells. Centrifuge at 300× g for 10 minutes. Aspirate the supernatant completely.
j. Resuspend cells in 3 mL of Cell isolation buffer per 1×107 cells.

Note: *For higher cell numbers, scale up the Cell isolation buffer volume accordingly. Generally, use 3-5 mL to resuspend cells for column loading. When using SOLIDEX®-ISOEx L Columns for cell depletion, resuspend up to 1.25 × 108 cells per 500 µL of Cell isolation buffer.*

D. Magnetic isolation

Place the Column on the magnet.

a. Rinse the Column once with 3 mL of Cell isolation buffer (SOLIDEX®-ISOEx L Column). Allow the buffer to run through, but do not let the Column run dry.
b. Transfer the cell suspension onto the prepared Column using a pipette. Collect the flow-through containing unlabeled cells.
c. Wash the Column with 3 mL × 3 of Cell isolation buffer. Collect the flow-through containing unlabeled cells in a suitable tube (e.g., 2 mL or 15 mL conical tube). Repeat the washing step twice. Add new Cell isolation buffer when the Column stops dripping but has not run completely dry.
d. Remove the Column from the magnet and place it into a suitable new collection tube (e.g., 15 mL or 50 mL conical tube).
e. Pipette 5 mL of Cell isolation buffer onto the Column. Immediately flush out the magnetically labeled cell fraction by firmly pushing the plunger into the Column.
f. Cells can be used for counting, purity analysis, or downstream applications. Removal of Nanobeads is not required. To ensure cell viability, immediately resuspend the target cell fraction in cell culture medium.

Notes

A. Avoid freezing during use and storage of the Nanobeads.
B. It is recommended to use low-binding pipette tips and centrifuge tubes to prevent loss of Nanobeads due to adsorption.
C. Before aspirating the Nanobeads, mix them gently. Avoid bubble formation during mixing.
D. This product is used for research use only.

Validation Data from GeneMedi

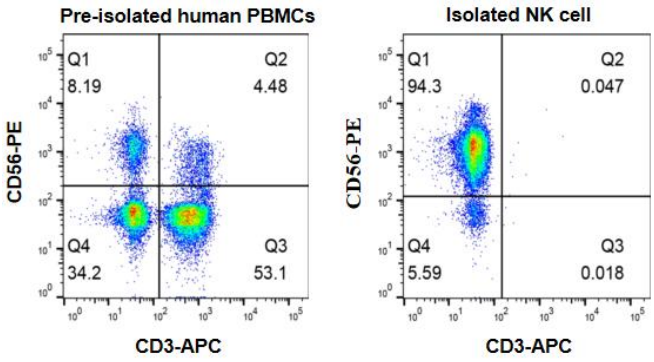

Figure 1. Flow cytometric analysis of enriched Human NK cells. Magnetic isolation was performed using SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based) and a biotinylated human Cocktail. Results show that the proportion of CD56+ NK cells was 8.19% before isolation and reached a purity of 94.3% after isolation.

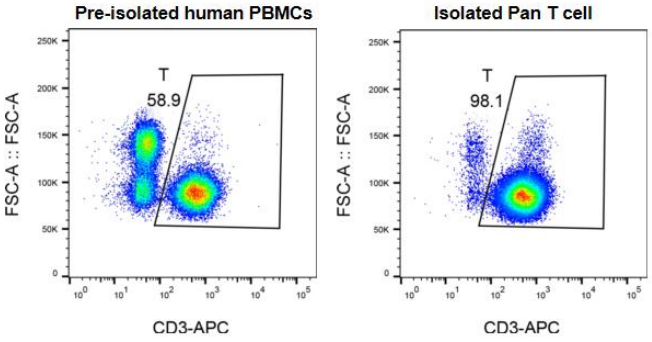

Figure 2. Flow cytometric analysis of enriched Human CD3+ T cells. Magnetic isolation was performed using SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based) and a biotinylated human Cocktail. Results show that the proportion of CD3+ T cells was 58.9% before isolation and reached a purity of 98.1% after isolation.

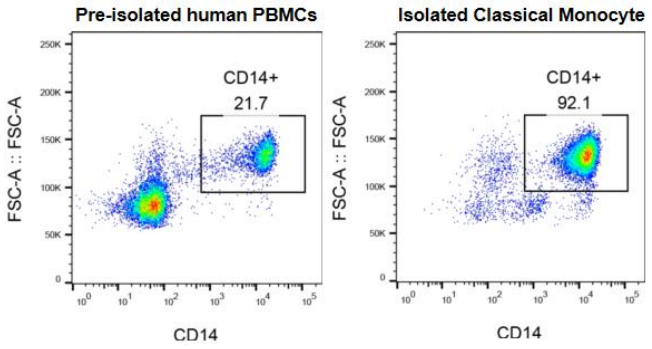

Figure 3. Flow cytometric analysis of enriched Human CD14+ Monocytes. Magnetic isolation was performed using SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based) and a biotinylated human Cocktail. Results show that the proportion of CD14+ cells was 21.7% before isolation and reached a purity of 92.1% after isolation.

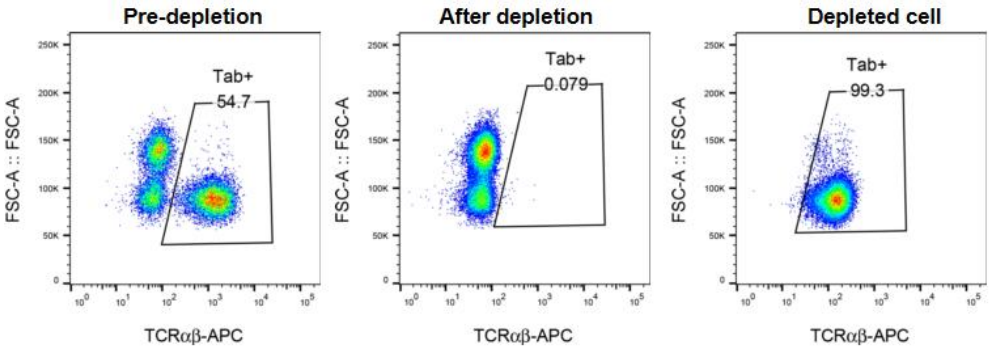

Figure 4. Flow cytometric analysis of depleted Human TCR αβ+ cells. Magnetic isolation was performed using SOLIDEX®-ISOEx anti-Biotin Nanobeads (Column-Based) and a biotinylated human TCR αβ antibody. Results show that after Column processing, the proportion of TCR αβ+ cells decreased from 54.7% to 0.079%, indicating a depletion efficiency of over 99.3%.